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=> s truncated plasmin proteolytic protein
L1             0 TRUNCATED PLASMIN PROTEOLYTIC PROTEIN

=> s plasmin fragment
L2           113 PLASMIN FRAGMENT

=> s l2 and "34 kDa"
L3             0 L2 AND "34 KDA"

=> s l2 and plasminogen
L4            28 L2 AND PLASMINOGEN

=> s l4 and "rPAI-123"
L5             0 L4 AND "RPAI-123"

=> dup remove l4
PROCESSING COMPLETED FOR L4
L6            13 DUP REMOVE L4 (15 DUPLICATES REMOVED)

=> d l6 1-13 cbib abs

L6   ANSWER 1 OF 13  CAPLUS  COPYRIGHT 2005 ACS on STN
2004:531314   Document No. 141:82302  Antisense oligonucleotides and siRNA to
     plasminogen receptor P11 mRNA for inhibiting tumor growth and
     metastasis.  Waisman, David (Mediomics, Llc, USA).  PCT Int. Appl. WO
     2004054517 A2 20040701, 140 pp.  DESIGNATED STATES: W:  AE, AG, AL, AM,
     AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE,
     DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN,
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IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US40029 20031212. PRIORITY: US 2002-2002/PV433140 20021213.

AB The present invention provides antisense oligonucleotides and siRNA to **plasminogen** receptor P11 mRNA for inhibiting tumor growth and metastasis. P11 protein is demonstrated to affect plasmin production and activity, MMP activity, **plasminogen** activation, antiangiogenic **plasmin fragment** production, cell invasion, tumor development and metastasis. Compns. that modulate levels of p11 either up or down are demonstrated to be effective in reducing tumor development. Also disclosed are cancer treatment methods that employ compns. that modulate p11 activity and clonal cell lines and assays useful for the identification of compns. that modulate p11 activity.

L6 ANSWER 2 OF 13 CAPLUS COPYRIGHT 2005 ACS on STN

2004:589236 Document No. 141:134059 Antisense oligonucleotides and siRNA to **plasminogen** binding/activating annexin II subunit p11 mRNA for inhibiting tumor growth and metastasis. Waisman, David M. (Can.). U.S. Pat. Appl. Publ. US 2004142897 A1 20040722, 66 pp., Cont.-in-part of U.S. Ser. No. 304,287. (English). CODEN: USXXCO. APPLICATION: US 2003-735577 20031212. PRIORITY: US 2001-2001/PV33386U 20011128; US 2002-2002/304287 20021126; US 2002-2002/PV433140 20021213.

AB The present invention provides antisense oligonucleotides and siRNA to annexin II subunit p11 mRNA for inhibiting tumor growth and metastasis. The p11 protein is demonstrated to affect plasmin production and activity, MMP activity, **plasminogen** activation, antiangiogenic **plasmin fragment** production, cell invasion, tumor development and metastasis. Compns. that modulate levels of p11 either up or down are demonstrated to be effective in reducing tumor development. Also disclosed are cancer treatment methods that employ compns. that modulate p11 activity and clonal cell lines and assays useful for the identification of compns. that modulate p11 activity.

L6 ANSWER 3 OF 13 CAPLUS COPYRIGHT 2005 ACS on STN

2003:334637 Document No. 138:348711 Anti-angiogenesis **plasmin fragment**, methods, compositions and uses therefor. Waisman, David; Kwon, Mijung (Can.). U.S. Pat. Appl. Publ. US 2003083234 A1 20030501, 29 pp. (English). CODEN: USXXCO. APPLICATION: US 2002-304287 20021126. PRIORITY: US 2001-PV333866 20011128.

AB This invention relates generally to methods of producing peptide-based anti-angiogenesis compds. using plasmin reductases, and specifically to methods of producing an A61 anti-angiogenic **plasmin fragment** using an annexin II heterotetramer or subunit thereof. This invention also relates to anti-angiogenesis methods and compns. comprising a plasmin reductase or polynucleotides encoding subunits thereof. Compns. useful for the inhibition or promotion of angiogenesis are also disclosed.

L6 ANSWER 4 OF 13 MEDLINE on STN DUPLICATE 1

2002179447. PubMed ID: 11781322. Identification of annexin II heterotetramer as a plasmin reductase. Kwon Mijung; Caplan Jennifer F; Filipenko Nolan R; Choi Kyu-Sil; Fitzpatrick Sandra L; Zhang Libo; Waisman David M. (Cancer Biology Research Group, Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta T2N 4N1, Canada.) Journal of biological chemistry, (2002 Mar 29) 277 (13) 10903-11. Electronic Publication: 2002-01-07. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Annexin II heterotetramer (AIIt) is a Ca(2+)- and phospholipid-binding protein that consists of two copies of a p36 and p11 subunit. AIIt regulates the production and autoproteolysis of plasmin at the cell surface. In addition to its role as a key cellular protease, plasmin also

plays a role in angiogenesis as the precursor for antiangiogenic proteins. Recently we demonstrated that the primary antiangiogenic **plasmin fragment**, called A(61) (Lys(78)-Lys(468)) was released from cultured cells. In the present study we report for the first time that AIIt possesses an intrinsic plasmin reductase activity. AIIt stimulated the reduction of the plasmin Cys(462)-Cys(541) bond in a time- and concentration-dependent manner, which resulted in the release of A(61) from plasmin. Mutagenesis of p36 C334S and either p11 C61S or p11 C82S inactivated the plasmin reductase activity of the isolated subunits, suggesting that specific cysteinyl residues participated in the plasmin reductase activity of each subunit. Furthermore, we demonstrated that the loss of AIIt from the cell surface of HT1080 cells transduced with a retroviral vector encoding p11 antisense dramatically reduced the cellular production of A(61) from **plasminogen.** This is the first demonstration that AIIt regulates the cellular production of the antiangiogenic **plasminogen** fragment, A(61).

L6 ANSWER 5 OF 13 MEDLINE on STN DUPLICATE 2
2001236133. PubMed ID: 11114303. Purification and characterization of A61. An angiostatin-like **plasminogen** fragment produced by plasmin autodigestion in the absence of sulfhydryl donors. Kassam G; Kwon M; Yoon C S; Graham K S; Young M K; Gluck S; Waisman D M. (Cancer Biology Research Group, Department of Biochemistry, University of Calgary, Calgary, Alberta T2N 4N1, Canada.) Journal of biological chemistry, (2001 Mar 23) 276 (12) 8924-33. Electronic Publication: 2000-12-12. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Plasmin, a broad spectrum proteinase, is inactivated by an autoproteolytic reaction that results in the destruction of the heavy and light chains of the protein. Recently we demonstrated that a 61-kDa **plasmin fragment** was one of the major products of this autoproteolytic reaction (Fitzpatrick, S. L., Kassam, G., Choi, K. S., Kang, H. M., Fogg, D. K., and Waisman, D. M. (2000)Biochemistry 39, 1021-1028). In the present communication we have identified the 61-kDa **plasmin fragment** as a novel four kringle-containing protein consisting of the amino acid sequence Lys(78)-Lys(468). To avoid confusion with the plasmin(ogen) fragment, angiostatin(R) (Lys(78)-Ala(440)), we have named this protein A(61). Unlike angiostatin, A(61) was produced in vitro from plasmin autodigestion in the absence of sulfhydryl donors. A(61) bound to lysine-Sepharose and also underwent a large increase in fluorescence yield upon binding of the lysine analogue, trans-4-aminomethylcyclohexanecarboxylic acid. Circular dichroism suggested that A(61) was composed of 21% beta-strand, 14% beta-turn, 18% 3(1)-helix and 8% 3(10)-helix. A(61) was an anti-angiogenic protein as indicated by the inhibition of bovine capillary endothelial cell proliferation. **Plasminogen** was converted to A(61) by HT1080 cells and bovine capillary endothelial cells. Furthermore, a **plasminogen** fragment similar to A(61) was present in the serum of humans as well as normal and tumor-bearing mice. These results establish that plasmin turnover can generate anti-angiogenic **plasmin fragments** in a nonpathological setting.

L6 ANSWER 6 OF 13 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2001:305607 Document No.: PREV200100305607. Unusually severe thrombophilia in dysplasminogenemia: Profound disruption of plasmin generation caused by a novel L696P amino acid substitution in the enzymatically active alpha-helix. Baumann-Baretti, B. [Reprint author]; Vetter, B. [Reprint author]; Kiesewetter, H. [Reprint author]; Ziemer, S. [Reprint author]; Kulozik, A. E. [Reprint author]. Charite Medical Center, Humboldt-University, Berlin, Germany. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 52a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology. San Francisco, California, USA. December 01-05, 2000. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB The role of dysplasminogenemia in thrombophilia is variable but most forms remain asymptomatic. Rarely, the reduction of **plasminogen**

activity is associated with recurrent venous thrombosis. We describe here a novel dysfunctional **plasminogen** (plg) in a family with severe thrombophilia. The 59 year old proposita had suffered from one postoperative deep vein thrombosis at the age of 28. Her mother and brother suffered from deep vein thrombosis and fatal pulmonary embolism. The thrombophilia workup identified a normal **plasminogen** level of 14.6 mg/dl (n: 6-25 mg/dl) but a reduced chromogenic activity of 49% in the proposita and her asymptomatic son (15.3 mg/dl; 42%). After t-PA and u-PA stimulation the fibrinolytic capacity in unmodified and in dextransulfate activated plasma was reduced to 19% (n: 70-130%) and 45% (n: 70-130%), respectively. DNA sequence analysis of the plg-gene demonstrated a heterozygous genotype for a novel CTT>CCT mutation of codon 696, which predicts a Leu>Pro amino acid substitution at this position (L696P). This mutation affects the enzymatically active **plasmin fragment** at its structurally and functionally critical C-terminal alpha-helix. The introduction of a novel Pro residue at this position is likely to severely disrupt helix formation, which explains the profound functional and clinical effect of this mutation.

L6 ANSWER 7 OF 13 CAPLUS COPYRIGHT 2005 ACS on STN
1998:239304 Document No. 128:294008 Fragments of **plasminogen** effective in inhibiting tumor metastasis and growth and process for preparing the same. Morikawa, Wataru; Miyamoto, Seiji (Juridical Foundation the Chemo-Sero-Therapeutic Research Institute, Japan; Morikawa, Wataru; Miyamoto, Seiji). PCT Int. Appl. WO 9815643 A1 19980416, 34 pp. DESIGNATED STATES: W: AU, CA, KR, US; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (Japanese). CODEN: PIXXD2. APPLICATION: WO 1997-JP3635 19971009. PRIORITY: JP 1996-287651 19961009.

AB Fragments of a **plasminogen** effective in inhibiting tumor metastasis and growth, an enzymic process for preparing the fragments, and a tumor metastasis and growth inhibitor containing the fragments as the active ingredient are presented. The fragments are obtained from the elastase-induced hydrolysis product of Lys-**plasminogen** that is obtained by treating a **plasminogen** with plasmin and that preferably has a potent heparin-binding activity. Alternatively, the Lys-**plasminogen** is prepared by autolysis of **plasminogen** in the presence of tranexamic acid. The inhibitor is useful for clin. therapy of solid cancers typified by lung and colon cancers.

L6 ANSWER 8 OF 13 MEDLINE on STN DUPLICATE 3
1999030426. PubMed ID: 9813061. Macrophage formation of angiostatin during inflammation. A byproduct of the activation of **plasminogen.**
Falcone D J; Khan K M; Layne T; Fernandes L. (Department of Pathology, Cornell Medical College, New York, New York 10021, USA.. dfalcone@mail.med.cornell.edu) . Journal of biological chemistry, (1998 Nov 20) 273 (47) 31480-5. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Angiostatin is a potent inhibitor of tumor angiogenesis and the growth of metastatic foci. Recent studies have indicated that neoplastic cells can generate angiostatin directly or in cooperation with tumor-associated macrophages. In studies reported here, we determined whether angiostatin is generated in mice under non-neoplastic settings. Utilizing murine RAW264.7 macrophages and thioglycollate-elicited peritoneal macrophages, we demonstrate that angiostatin-like fragments are generated as a byproduct of the proteolytic regulation of membrane-bound plasmin. Plasmin proteolysis and subsequent loss in membrane-bound plasmin activity requires active plasmin but was unaffected by inhibitors of metalloproteinases. Lysine binding fragments of plasmin, isolated from macrophage-conditioned media utilizing affinity chromatography, appeared as a major (48 kDa) and two minor bands (42 and 50 kDa) in SDS-polyacrylamide gel electrophoresis and were immunoreactive with anti-kringle 1-3 IgG. Each peptide begins with Lys77 and contains the entire sequence of angiostatin. The affinity isolated **plasmin fragments** inhibited bFGF-induced endothelial cell proliferation. Lavage fluid recovered from the peritoneal cavities of mice previously

injected with thioglycollate contained angiostatin-like **plasmin fragments** similar to those generated in vitro. This is the first demonstration that angiostatin-like **plasmin fragments** are generated in a non-neoplastic inflammatory setting. Thus, in addition to regulating pericellular plasmin activity, proteolysis of plasmin generates inactive kringle-containing fragments expressing angiostatic properties.

L6 ANSWER 9 OF 13 CAPLUS COPYRIGHT 2005 ACS on STN
1984:172254 Document No. 100:172254 Demonstration of 99mTc-labeled plasmin, K1 + 2 + 3, K4 and 'mini-**plasminogen**' on ex vivo thrombi. Hedner, U.; Tengborn, L. (Dep. Coagulation Disord., Univ. Lund, Lund, Swed.). Progress in Fibrinolysis, 6, 449-52 (English) 1983. CODEN: PFIBDA. ISSN: 0262-0790.
AB Plasmin, both human and porcine, and various human **plasminogen/plasmin fragments** [K1+2+3 (amino acid residues 73/79-353), K4 (amino acids 354-439), and mini-**plasminogen** (amino acids 442-790)] produced by elastase proteolysis bound to the surface of a preformed thrombus. This binding occurred even in the presence of free α2-antiplasmin. Tranexamic acid (1, 10, or 12 mM) did not interfere with the binding, indicating that the fragments have a binding specific for fibrin which is different from lysine binding sites. Since all the **plasminogen/plasmin fragments** bound to the thrombus, the binding site may be located in a structure common to the different kringle parts of the mol.

L6 ANSWER 10 OF 13 MEDLINE on STN DUPLICATE 4
83075471. PubMed ID: 6216916. Fibrinolysis and fibrinogenolysis by Val442-plasmin. Ney K A; Pizzo S V. Biochimica et biophysica acta, (1982 Nov 9) 708 (2) 218-24. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.
AB Elastase cleavage of Lys77-plasmin results in the formation of Val442-plasmin. This result suggests that small, active **plasmin fragments** can be produced even under conditions of high **plasminogen** activator levels such as occur in vivo. We examined the effect of the generation of such fragments by studying the degradation of fibrinogen and fibrin by Val442-plasmin. Val442-plasmin lysis of fibrinogen yielded the same products as obtained with Lys77-plasmin, but at a slightly lower rate. Lysine inhibited fibrinogenolysis by both Lys77-plasmin and Val442-plasmin. The marked inhibition observed at concentrations higher than 10 mM lysine occurred to the same extent for both proteases. In addition, the products and rate of fibrinolysis were the same for both proteases. These results indicate that the lysine binding regions present in Lys77-plasmin but absent in Val442-plasmin do not determine the rate, reaction products, or lysine inhibition of fibrinolysis and fibrinogenolysis by plasmin.

L6 ANSWER 11 OF 13 CAPLUS COPYRIGHT 2005 ACS on STN
1980:442599 Document No. 93:42599 Activation of human neo-**plasminogen**-Val442 by urokinase and streptokinase and a kinetic characterization of neo-plasmin-Val442. Powell, James R.; Castellino, Francis J. (Dep. Chem., Univ. Notre Dame, Notre Dame, IN, 46556, USA). Journal of Biological Chemistry, 255(11), 5329-35 (English) 1980. CODEN: JBCHA3. ISSN: 0021-9258.
AB The activation properties of a low-mol.-weight form of human **plasminogen**, neo-**plasminogen**-Val442 (I), by urokinase and streptokinase (II) were examined Despite the elimination of .apprx.60% of the total mass of native **plasminogen** in I, the latter mol. remained a zymogen, with activation capabilities not very different from the native mol. In addition, the stoichiometric II-neo-plasmin-Val442 (III) complex served as a very efficient activator of species of **plasminogen** which were not activated by II alone. The kinetic properties of III produced by activation of I with catalytic levels of urokinase and II, as well as the stoichiometric II-III complex, were evaluated. The Km for Nα-tosyl-L-arginine Me ester with III was 4.0

mM, and the value for the II-III complex was 2.4 mM. The kcat values for this substrate with each enzyme species were 45.0 and 30.1 s-1, resp. The Ki value for the competitive inhibitor, benzamidine, was 1.3 mM for III and 0.5 mM for its stoichiometric complex with II. Pre-steady state kinetic studies utilizing p-nitrophenyl-p'-guanidinobenzoate revealed a k2 of 0.75 s-1 for III and 0.57 for the stoichiometric II-III complex. The corresponding Ks values for this substrate were 99.3 μM and 37.2 μM, resp.

L6 ANSWER 12 OF 13 CAPLUS COPYRIGHT 2005 ACS on STN
1979:199568 Document No. 90:199568 Enzymic properties of the neo-plasmin-Val-442 (miniplasmin). Christensen, Ulla; Sottrup-Jensen, Lars; Magnusson, Staffan; Petersen, Torben E.; Clemmensen, Inge (Chem. Lab. IV, Univ. Copenhagen, Copenhagen, Den.). Biochimica et Biophysica Acta, 567(2), 472-81 (English) 1979. CODEN: BBACAQ. ISSN: 0006-3002.
AB The enzymic properties of the activated **plasminogen** fragment (Val-442-Arg-560; SS-bridged to Val-561-Asn-790), also called miniplasmin or neoplasmin-Val-442, were studied. Neoplasmin was prepared by urokinase-catalyzed conversion of the corresponding fragment of **plasminogen** (Val-442-Asn-790) produced by specific limited proteolysis of native **plasminogen** by porcine pancreatic elastase and purified by chromatog. on L-lysine-Sepharose 4B. The kinetic parameters of hydrolysis of a number of synthetic substrates by plasmin and miniplasmin were alike. Furthermore, inhibition by 6-aminohexanoic acid and the pH dependence of benzoylarginine Et ester hydrolysis were identical for the 2 enzymes. Thus, the catalytic site of miniplasmin is very similar to that of plasmin. The interaction of miniplasmin with α2-antiplasmin was studied in the presence and absence of 6-aminohexanoic acid. The reaction scheme for the reaction of plasmin with the inhibitor also fit the reaction of miniplasmin with the inhibitor. However, miniplasmin initially reacted with the inhibitor less readily than did plasmin, but the rate of the 2nd reaction step was equal to that of the corresponding step in the inhibitor-plasmin reaction. The hypothesis that a site(s) other than the catalytic site of plasmin located at the N-terminal part of the heavy chain (residues 77-441) primarily dets. the association rate of plasmin and α2-antiplasmin is supported.

L6 ANSWER 13 OF 13 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
78057355 EMBASE Document No.: 1978057355. Fragmentation of porcine β lipotropic hormone with plasmin. Graf L.. Res. Inst. Pharmaceut. Chem., Budapest, Hungary. Acta Biochimica et Biophysica Academiae Scientiarum Hungaricae Vol. 11, No. 4, pp. 267-277 1976.
CODEN: ABBPAP
Language: English.
AB Porcine β-lipotropic hormone was digested by plasmin and the fragments released were isolated and identified. Based on the structure and the yield of the **plasmin fragments** and also on the time-course of the digestion, the relative sensitivity of the theoretically susceptible bonds towards the action of plasmin was estimated. The Lys(79)-Asn(80) bond was the most susceptible to plasmin, the Arg(51)-Trp(52) and Arg(60)-Tyr(61) bonds were split to a lesser extent than the Lys(79)-Ar(80) bond, but much faster than the Lys(46)-Met(47), Lys(69)-Ser(70) and Lys(84)-Asn(85) bonds of the β-lipotropic hormone structure. Further lysyl and arginyl bonds of the molecule were not attacked under our experimental conditions.

=> s plasminogen
L7 164328 PLASMINOGEN

=> s l7 and react
L8 763 L7 AND REACT

=> s l8 and truncated plasminogen activator inhibitor type I

L9 0 L8 AND TRUNCATED PLASMINOGEN ACTIVATOR INHIBITOR TYPE I

=> s l8 and "rPAI-1"
L10 1 L8 AND "RPAI-1"

=> d l10 cbib abs

L10 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2005 ACS on STN
2003:680380 Document No. 139:391890 Expression, purification of Lys10-PAI-1 in E. coli and its gel retardation experiments. Sun, Xing-hui; Li, Ping; Zhang, Yu-qing; Tan, Li; Wang, Xia; Hou, Min; Chen, Yi-yun; Zhu, Yun-song (Dep. Mol. Genetics, Med. Sch. of Fudan Univ., Shanghai, 200032, Peop. Rep. China). Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao, 19(4), 457-462 (Chinese) 2003. CODEN: ZSHXF2. ISSN: 1007-7626. Publisher: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui.
AB The fused gene (Lys10-PAI-1) of ten lysine and **plasminogen** activator inhibitor-I was cloned into prokaryotic expression vector pET32a(+) and pET28a(+). The recombinant expression plasmid pET32-PAI and pET28-PAI were transformed into E. coli BL21 (DE3). After induction for 3 h by IPTG, the expression recombinants showed a high level expression of recombinant protein with both Trx·PAI-1 and **rPAI-1** being more than 20% of the total bacterial protein. SDS-PAGE and Western blot anal. indicated that the mol. weight of Trx PAI-1 and **rPAI-1** was about 63 kD and 43 kD, resp. and the recombinant proteins could **react** specifically with anti-PAI-I antibody. The purified recombinant Trx·PAI-1 and rPAI-I were obtained after denaturation, renaturation, dialysis and affinity chromatog. In gel retardation experiment, the purified protein retarded plasmid in gel electrophoresis, and obviously changed the mobility of plasmid. These results indicate that Trx·PAI-1 and rPAI-1 can bind plasmid, and are hopeful to be used to transfer gene into cells.

=> s "plasminogen" and "uPA"
L11 11978 "PLASMINOGEN" AND "UPA"

=> s l11 and plasmin
L12 2457 L11 AND PLASMIN

=> s l12 and fragment
L13 266 L12 AND FRAGMENT

=> s l13 and weight
L14 51 L13 AND WEIGHT

=> s l14 "34 kDa"
MISSING OPERATOR L14 "34
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

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PROCESSING COMPLETED FOR L14
L15 17 DUP REMOVE L14 (34 DUPLICATES REMOVED)

=> d l15 1-17 cbib abs

L15 ANSWER 1 OF 17 EMBASE
on STN DUPLICATE 1
2004178495 EMBASE Fibrinogen catabolism within the procoagulant VX-2 tumor of rabbit lung in vivo: Effluxing fibrin(ogen) **fragments** contain antiangiogenic activity. Hatton M.W.C.; Southward S.M.R.; Legault K.J.; Ross B.L.; Clarke B.J.; Bajzar L.; Blajchman M.A.; Singh G.; Richardson M.. Dr. M.W.C. Hatton, Dept. of Pathol. and Molec. Medicine, McMaster Univ. Hlth. Sciences Centre, 1200 Main St W, Hamilton, Ont. L8N 3Z5, Canada. hattonm@mcmaster.ca. Journal of Laboratory and Clinical Medicine

Vol. 143, No. 4, pp. 241-254 2004.
Refs: 41.
ISSN: 0022-2143. CODEN: JLCMAK
Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 20040513
AB Many types of solid tumors are known to be procoagulant environments. This is partly because a hyperpermeable vascular system within the tumor allows plasma hemostatic factors to accumulate in relatively high concentrations in the stroma, and many solid-tumor cells express tissue factor or a procoagulant factor. These circumstances appear to exist in the VX-2 lung tumor of the New Zealand White (NZW) rabbit, and they sustain a measurable turnover of stromal deposits of fibrin(ogen). We have measured the turnover of fibrinogen within tumors of the VX-2 tumor-burdened rabbit and analysed the catabolic products of fibrin(ogen) and the status of fibrinolysis in tumor-derived interpleural effusate. Using intravenously injected (125)I-labeled rabbit fibrinogen as a marker, we found that fibrinogen (approximate blood concentration 1740 μg/mL) passed from blood to VX-2 tumor stroma, saturating the tumor at a concentration of approximately 348 μg fibrinogen/g in approximately 12 hours. We measured fibrin(ogen) **fragments**, at a concentration of approximately 292 μg/ml, in interpleural effusates that we recovered from 13% of the VX-2-burdened rabbits. Unreduced fibrin(ogen) **fragments** consisted of 4 major components with a relative molecular mass of approximately 250,000 (assumed to be **fragment** X; approximately 9% of total **fragments** from densitometry of immunoblots), 200,000 (D-dimer; 41%), 110,000 (**fragment** D; 49%), and 50,000 to 55,000 (**fragment** E; 1%-2%) kD. Total fibrin(ogen) **fragments** immunopurified from effusates exhibited an antiangiogenic effect when subjected to a chick embryo chorioallantoic membrane procedure. Interpleural effusates were devoid of **plasmin** activity or active **plasminogen** activator inhibitor-1 but contained **plasmin** complexes and active urokinase-like **plasminogen** activator (**uPA**), α(2)-antiplasmin, and thrombin-activatable fibrinolysis inhibitor. We speculate that VX-2 cells release **uPA** to activate fibrinolysis within the tumor stroma. Catabolic products of hemostasis (eg, fibrinolytic **fragments**, angiostatin) flux from the stroma into the interpleural space, thereby providing a net antiangiogenic property to the effusate and ultimately to the lymphatic and circulatory systems.

L15 ANSWER 2 OF 17 MEDLINE on STN
2002369602. PubMed ID: 12114193. Urokinase induces its own expression in Beas2B lung epithelial cells. Shetty Sreerama; Pendurthi Usha R; Halady Prathap Kumar Shetty; Azghani Ali O; Idell Steven. (Department of Medical Specialties, The University of Texas Health Center at Tyler, Tyler, Texas 75708, USA.. sreerama.shetty@uthct.edu) . American journal of physiology. Lung cellular and molecular physiology, (2002 Aug) 283 (2) L319-28. Journal code: 100901229. ISSN: 1040-0605. Pub. country: United States. Language: English.
AB The urokinase-type **plasminogen** activator (**uPA**) interacts with its receptor (uPAR) to promote local proteolysis as well as cellular proliferation and migration. These functions contribute to the pathogenesis of lung inflammation and remodeling as well as the growth and invasiveness of lung neoplasms. In this study, we sought to determine if **uPA** alters its own expression in lung epithelial cells. Using immunoprecipitation and Western and Northern blotting techniques, we found that **uPA** treatment enhanced **uPA** expression in Beas2B lung epithelial cells in a time- and concentration-dependent manner. The induction of **uPA** expression is mediated through its cell surface receptor uPAR and does not require **uPA** enzymatic activity. The amino-terminal **fragment** of **uPA**, lacking the catalytic domain, is sufficient to induce **uPA** expression. The serine protease **plasmin** and the protease inhibitor aprotinin failed to alter **uPA**-mediated **uPA** expression, whereas alpha-thrombin potentiated the response. Pretreatment of Beas2B cells

with a tyrosine kinase inhibitor, herbimycin, suggests that activation of tyrosine kinase(s) is involved in the **uPA**-mediated **uPA** expression. Induction of **uPA** expression by exposure of lung-derived epithelial cells to **uPA** is a newly defined pathway by which this protease could influence expression of local fibrinolytic activity and other **uPA**-dependent cellular responses germane to lung inflammation or neoplasia.

L15 ANSWER 3 OF 17 MEDLINE on STN

2002426205. PubMed ID: 12183060. Urokinase is required for the formation of mactinin, an alpha-actinin **fragment** that promotes monocyte/macrophage maturation. Luikart Sharon; Masri Mohammed; Wahl Dan; Hinkel Tim; Beck James M; Gyetko Margaret R; Gupta Pankaj; Oegema Theodore. (Veterans Affairs Medical Center, Minneapolis, MN, USA.. sharon.luikart@med.va.gov) . Biochimica et biophysica acta, (2002 Aug 19) 1591 (1-3) 99-107. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB We have previously shown that lysates from HL-60 myeloid leukemia cells or from peripheral blood monocytes are able to degrade alpha-actinin to form a 31-kDa amino-terminal **fragment** with monocyte/macrophage maturation promoting activity. In contrast, intact alpha-actinin, which is a 100-kDa actin-binding protein, has no differentiating activity. The aim of this study was to investigate the enzyme responsible for the degradation of alpha-actinin to form this **fragment**, named mactinin. The ability of cell lysates to degrade [125I]alpha-actinin in the presence of various enzyme inhibitors, including inhibitors of metalloproteinases, cysteine proteinases, and serine proteases, was measured. Phenylmethylsulfonyl fluoride (PMSF) was the only inhibitor able to prevent formation of mactinin by cell lysate degradation of alpha-actinin, suggesting that a serine protease is responsible for the digestion. Of the various serine proteases tested (thrombin, **plasmin**, and urokinase), only urokinase was able to produce a 31-kDa band. The urokinase-generated 31-kDa band promoted maturation in HL-60 cells. Amiloride, a specific inhibitor of urokinase, inhibited production of the 31-kDa alpha-actinin **fragment** by HL-60 cell lysates. For in vivo tests, inflammatory fluid (from bronchoalvelolar lavage) was collected from **uPA** (urokinase) knockout mice and their wild-type counterparts after intratracheal challenge with Pneumocystis carinii. Although most (6 of 8) wild-type mice had mactinin in their inflammatory fluid samples, none (0 of 8) of the **uPA** knockout mice had mactinin present ($P<0.01$). These results demonstrate that urokinase is necessary and sufficient for the formation of the monocyte/macrophage maturation promoting **fragment**, mactinin, in vitro and in vivo. These findings support the role of urokinase in the regulation of monocyte/macrophage functions, such as that occurring in inflammatory reactions.

L15 ANSWER 4 OF 17 MEDLINE on STN DUPLICATE 2

2001376666. PubMed ID: 11342540. Urokinase induces expression of its own receptor in Beas2B lung epithelial cells. Shetty S; Idell S. (Department of Medical Specialties, The University of Texas Health Center at Tyler, Biomedical Research Bldg., 11937 U.S. Highway 271, Tyler, TX 75708, USA.. sreerama.shetty@uthct.edu) . Journal of biological chemistry, (2001 Jul 6) 276 (27) 24549-56. Electronic Publication: 2001-05-07. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Interaction between the urokinase-type **plasminogen** activator (**uPA**) and its receptor (uPAR) localizes cellular proteolysis and promotes cellular proliferation and migration. The interaction between **uPA** and uPAR at the surface of epithelial cells thereby contributes to the pathogenesis of lung inflammation and neoplasia. In this study, we sought to determine if **uPA** itself alters uPAR expression by lung epithelial cells. **uPA** enhanced uPAR expression as well as (125)I-**uPA** binding in Beas2B lung epithelial cells in a time- and concentration-dependent manner. The **uPA**-mediated induction of uPAR is not accomplished through its

receptor and requires enzymatic activity. The low molecular **weight fragment** of **uPA**, lacking the receptor binding domain, was as potent as intact two-chain **uPA** in inducing expression of uPAR at the cell surface. **Plasmin**, the end product of **plasminogen** activation, did not alter **uPA** -mediated uPAR expression. Induction of uPAR by **uPA** represents a novel pathway by which epithelial cells can regulate uPAR-dependent cellular responses that may contribute to stromal remodeling in lung injury or neoplasia.

L15 ANSWER 5 OF 17 MEDLINE on STN DUPLICATE 3
2001185431. PubMed ID: 11254548. **Plasminogen** binding and activation by Mycoplasma fermentans. Yavlovich A; Higazi A A; Rottem S. (Department of Membrane and Ultrastructure Research, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.) Infection and immunity, (2001 Apr) 69 (4) 1977-82. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The binding of **plasminogen** to Mycoplasma fermentans was studied by an immunoblot analysis and by a binding assay using iodine-labeled **plasminogen.** The binding of 125I-labeled **plasminogen** was inhibited by unlabeled **plasminogen**, lysine, and lysine analog epsilon-aminocaproic acid. Partial inhibition was obtained by a **plasminogen fragment** containing kringles 1 to 3 whereas almost no inhibition was observed with a **fragment** containing kringle 4. Scatchard analysis revealed a dual-phase interaction, one with a dissociation constant (kd) of 0.5 microM and the second with a kd of 7.5 microM. The estimated numbers of **plasminogen** molecules bound were calculated to be 110 and 790 per cell, respectively. Autoradiograms of ligand blots containing M. fermentans membrane proteins incubated with 125I-labeled **plasminogen** identified two **plasminogen** -binding proteins of about 32 and 55 kDa. The binding of **plasminogen** to M. fermentans enhances the activation of **plasminogen** to **plasmin** by the urokinase-type **plasminogen** activator (**uPA**), as monitored by measuring the breakdown of chromogenic substrate S-2251. Enhancement was more pronounced with the low-molecular-**weight** and the single-chain **uPA** variants, known to have low **plasminogen** activator activities. The binding of **plasminogen** also promotes the invasion of HeLa cells by M. fermentans. Invasion was more pronounced in the presence of **uPA**, suggesting that the ability of the organism to invade host cells stems not only from its potential to bind **plasminogen** but also from the activation of **plasminogen** to **plasmin.**

L15 ANSWER 6 OF 17 MEDLINE on STN DUPLICATE 4
1999272427. PubMed ID: 10339491. Characterization of cell-associated **plasminogen** activation catalyzed by urokinase-type **plasminogen** activator, but independent of urokinase receptor (uPAR, CD87). Longstaff C; Merton R E; Fabregas P; Felez J. (The National Institute for Biological Standards and Control, South Mimms, Hertfordshire, UK.. clongstaff@nibsc.ac.uk) . Blood, (1999 Jun 1) 93 (11) 3839-46. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB The 55-kD urokinase (**uPA**) receptor (uPAR, CD87) is capable of binding **uPA** and may be involved in regulating cell-associated **plasminogen** activation and pericellular proteolysis. While investigating the relationship between uPAR levels and **plasmin** generation, we found that **uPA**-catalyzed **plasminogen** activation is stimulated by cells which do not express uPAR. This uPAR-independent mechanism appears to be at least as effective in vitro as uPAR-dependent stimulation, such that stimulation on the order of 30-fold was observed, resulting from improvements in both apparent kcat and apparent Km. The mechanism depends on simultaneous binding of both **uPA** and **plasminogen** to the cell and requires the presence of the amino-terminal **fragment** (ATF), available in

single chain and two chain high-molecular-**weight uPA**, but not low-molecular-**weight uPA**. Stimulation was observed in all leukemic cell lines investigated at similar optimum concentrations of 10(6) to 10(7) cells/mL and may be more general. A mechanism is proposed whereby **uPA** can associate with binding sites on the cell surface of lower affinity, but higher capacity than uPAR, but these are sufficient to stimulate **plasmin** generation even at subphysiologic **uPA** concentrations. This mechanism is likely to operate under conditions commonly used for in vitro studies and may have some significance in vivo.

L15 ANSWER 7 OF 17 MEDLINE on STN DUPLICATE 5

1999065689. PubMed ID: 9848876. Urokinase receptor-dependent upregulation of smooth muscle cell adhesion to vitronectin by urokinase. Chang A W; Kuo A; Barnathan E S; Okada S S. (University of Pennsylvania School of Medicine, Philadelphia, USA.) Arteriosclerosis, thrombosis, and vascular biology, (1998 Dec) 18 (12) 1855-60. Journal code: 9505803. ISSN: 1079-5642. Pub. country: United States. Language: English.

AB The **plasminogen** activator system has been implicated in the modulation of the response to vascular injury. Although urokinase-type **plasminogen** activator (**uPA**) and its receptor (uPAR) may enhance matrix degradation as well as migration and invasion by smooth muscle cells (SMCs), their roles in cell adhesion are uncertain. Therefore, we examined the ability of **uPA** and uPAR to modulate adhesion of cultured human vascular SMCs to various matrices. We demonstrated a dose-dependent stimulation of adhesion by single-chain **uPA** (scuPA) to vitronectin (maximum 1.55-fold [+/-0. 04-fold] increase, 10 nmol/L, P<0.002) but not to laminin, collagen I, or collagen IV. Baseline adhesion to vitronectin was completely inhibited by both EDTA and RGD peptide but was restored to >40% of control in the presence of scuPA (P=0.001 and 0.046, respectively). Adhesion to vitronectin was also significantly enhanced by the amino-terminal **fragment** of **uPA** (P=0.007) and two-chain, high-molecular-**weight** **uPA** (P<0.01) but not by the low-molecular-**weight** **fragment** of **uPA**, which lacks the receptor-binding domain. Aprotinin, a **plasmin** inhibitor, had no effect on baseline or scuPA-stimulated adhesion, suggesting a **plasmin** -independent process. Preincubation of scuPA with soluble uPAR inhibited scuPA stimulation of adhesion by 88+/-14% (P=0.01), as did pretreatment of SMCs with phosphatidylinositol-specific phospholipase C, which removes glycophosphatidylinositol-anchored proteins, including uPAR. Antibodies to both alphavbeta3 and alphavbeta5 integrin inhibited baseline adhesion but not scuPA stimulation. Finally, coating plates with scuPA alone enabled cell adhesion, which could be inhibited by both soluble uPAR and anti-uPAR antibodies. These data suggest that **uPA** stimulates adhesion of SMCs specifically to vitronectin and that it is mediated by an interaction with uPAR. Upregulation of both proteins after vascular injury may facilitate migration through stimulation of both matrix degradation and cell adhesion.

L15 ANSWER 8 OF 17 MEDLINE on STN DUPLICATE 6

97010891. PubMed ID: 8857924. Contrasting effects of **plasminogen** activators, urokinase receptor, and LDL receptor-related protein on smooth muscle cell migration and invasion. Okada S S; Grobmyer S R; Barnathan E S. (University of Pennsylvania School of Medicine, Philadelphia 19104-6060, USA.) Arteriosclerosis, thrombosis, and vascular biology, (1996 Oct) 16 (10) 1269-76. Journal code: 9505803. ISSN: 1079-5642. Pub. country: United States. Language: English.

AB Smooth muscle cell (SMC) migration is an early response to vascular injury and contributes to the development of intimal thickening. Upregulation of several components of the **plasminogen** activator (PA) system has been documented after vascular injury. Utilizing a Transwell filter assay system and human umbilical vein SMCs, we sought to define the role of four different PA system components on SMC migration and matrix invasion: (1) PAs, (2) **plasmin**, (3) PA receptors, and (4) PA clearance

receptors (ie, low density lipoprotein receptor-related protein [LRP]). Addition of active two-chain urokinase-type PA (**UPA**) stimulated random migration (192 +/- 30% of control, 0.36 nmol/L, P < .001). The stimulation was inhibited by pretreatment with diisopropylfluorophosphate, PA inhibitor type 1 (PAI-1), or aprotinin, a **plasmin** inhibitor. Augmented migration was also observed with either low-molecular-**weight UPA** or the amino terminal **fragment** of **UPA** (ATF), with the effects being additive. Stimulation by ATF alone, however, was not inhibited by aprotinin. The stimulatory effect was not specific for **UPA**, in that tissue-type PA (TPA) also increased migration (169 +/- 9% of control, 10 nmol/L, P < .001); the augmentation was inhibited by pretreatment with DFP, PAI-1, or aprotinin and was additive to the **UPA** effect. Antibodies to the **UPA** receptor but not 5'-nucleotidase (another glycosylphosphatidylinositol-anchored cell surface protein) inhibited baseline and **UPA**-stimulated migration. Similarly, both **UPA** and TPA stimulated invasion of a collagen gel; this augmentation was inhibited by aprotinin, whereas antibodies to the **UPA** receptor reduced baseline invasion. Finally, we tested whether inhibition of LRP function, which mediates internalization of PA/inhibitor complexes, affected either process. Both antibodies to LRP and recombinant receptor associated protein, a known inhibitor of ligand binding to the LRP, significantly inhibited migration but did not affect collagen gel invasion. These data demonstrate the ability of several components of the PA system to modulate SMC migration and invasion in vitro via **plasmin**-dependent and -independent mechanisms.

L15 ANSWER 9 OF 17 CAPLUS COPYRIGHT 2005 ACS on STN
1996:688650 Document No. 126:155537 Degradation of human plasma and extracellular matrix fibronectin by tissue type **plasminogen** activator and urokinase. Marchina, Eleonora; Barlati, Sergio (Division of Biology and Genetics, University of Brescia, Brescia, 25123, Italy). International Journal of Biochemistry & Cell Biology, 28(10), 1141-1150 (English) 1996. CODEN: IJBBFU. ISSN: 1357-2725. Publisher: Elsevier.

AB Fibronectins and **plasminogen** activators, both tissue and urokinase types, are involved in the physiopathol. degradation of the extracellular matrix. Previous reports indicate that fibronectin can be degraded by urokinase without **plasminogen.** Also, we have shown that tissue-type **plasminogen** activator can cleave fibronectin, without **plasminogen**, generating **fragments** of 30 and 220-250 kDa detectable by immunoblotting anal. A comparison with urokinase-induced degradation indicates that the cleavage sites are the same of both **plasminogen** activators. One is close to the carboxyl-terminal, disrupting the fibronectin dimeric structure, and one is near the amino-terminal, generating a 30 kDa **fragment.** In solution, the activity of tissue-type **plasminogen** activator was prevalent on the amino-terminal site, while urokinase activity was prevalent on the carboxyl-terminal site. On fibronectin immobilized onto a gelatin coated surface, only the 30 kDa **fragment** was released when treated with both **plasminogen** activators. **Plasminogen** activators also were active on fibronectin assembled into the extracellular matrix of cultured fibroblasts. Urokinase caused the complete disappearance of extracellular matrix fibronectin, together with the release of the 30 and 220-250 kDa fibronectin **fragments** , but left a flat morphol., while tissue-type **plasminogen** activator induced the release of the 30 kDa **fragment** associated with changes in cellular morphol. The **plasminogen**-independent fibronectin degradation exerted by tissue-type **plasminogen** activator and urokinase is 100 times lower than the exerted by **plasmin.** This may provide a mechanism for localized and limited degradation of fibronectin preventing the generalized proteolysis associated with **plasminogen** activation.

L15 ANSWER 10 OF 17 MEDLINE on STN DUPLICATE 7
96404689. PubMed ID: 8808830. Mechanism of tumor cell-induced

extracellular matrix degradation--inhibition of cell-surface proteolytic activity might have a therapeutic effect on tumor cell invasion and metastasis. Kobayashi H. (Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine.) Nippon Sanka Fujinka Gakkai zasshi, (1996 Aug) 48 (8) 623-32. Ref: 14. Journal code: 7505749. ISSN: 0300-9165. Pub. country: Japan. Language: Japanese.

AB Tumor cells produce urokinase-type **plasminogen** activator (**uPA**) in an enzymatically inactive proenzyme form (pro-**uPA**). Secreted pro-**uPA** can immediately bind to the specific **uPA** receptors (uPAR) on tumor cell surface with high affinity. The uPAR specifically recognizes enzymatically inactive pro-**uPA** and active high molecular **weight-uPA** (HMW-**uPA**) by their growth factor-like terminal domain. uPAR is a glycoprotein of approximately 55 kDa; the affinity for **uPA** is high (0.2 nM) and the rate of dissociation is low. Receptor-bound **uPA** catalizes the formation of **plasmin** on the cell surface to generate the proteolytic cascade that contributes to the breakdown of basement membrane and extracellular matrix. The plasma membrane uPAR has attracted considerable attention because of its role in migration and tissue invasion by mononuclear phagocytes and malignant cells. In some cell types uPAR localizes **uPA** to cell-cell and cell-substratum contact sites, providing the possibility of a directional proteolysis that may be involved in cell migration and invasion. Recently it has been reported that competitive displacement of **uPA** from uPAR resulted in decreased proteolysis, suggesting that the cell surface is the preferred site for **uPA**-mediated protein degradation. Various very different approaches to interfere with the expression or reactivity of **uPA** or uPAR at the gene or protein level were successfully tested including antisense oligonucleotides, antibodies, inhibitors and recombinant or synthetic **uPA** and uPAR analogues. Recently we have reported that a highly purified urinary trypsin inhibitor (UTI) efficiently inhibits soluble and tumor cell-surface receptor-bound **plasmin.** UTI inhibits not only tumor cell invasion in an in vitro assay but also production of experimental and spontaneous lung metastasis in an in vivo mouse model. The anti-invasive effect is dependent on the anti-**plasmin** activity of UTI. UTI peptide, which inhibits **plasmin** activity, synthesized by an automated peptide synthesizer showed mouse 3LL cell invasion inhibitory activity. UTI and the effective peptide inhibited tumor cell invasion through Matrigel. UTI did not inhibit tumor cell proliferation or the binding of the cells to Matrigel. Also, UTI did not inhibit chemotactic migration of tumor cells to fibronectin. It is likely that UTI acts as a protease inhibitor. We attempted to synthesize conjugates between ATF and UTI. Thus, conjugating a physiological **plasmin** inhibitor to ATF might target it to reduce cell-associated proteolytic activity to the close environment of the uPAR-expressing tumor cell surface and subsequently may effectively inhibit tumor cell invasion and metastasis, because the cell surface uPAR might be a critical component of the metastatic machinery. A method of conjugation of the UTI domain II (HI-8), to the receptor-binding amino-terminal **fragment** (ATF) of **uPA** has been developed utilizing the heterobifunctional cross-linking reagent, N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP). The conjugate retained its protease inhibiting activity and showed a binding reactivity to uPAR on the surface of tumor cells. We have shown that the conjugate exhibits **plasmin** inhibition to the close environment of the cell surface and subsequently inhibits the tumor cell invasion through Matrigel in an in vitro invasion assay. In order to extend our idea, we attempt to produce a novel hybrid molecule consisting of the ATF of **uPA** placed at the N-terminus of UTI domain II (HI-8) by protein engineering techniques. Exogenously applied ATFHI hybrid protein can immediately bind to the specific uPAR on cell surfaces with high affinity. The receptor-bound hybrid protein focuses the protease-inhibiting activity to the tumor cell surface. This is effectively a bifunctional molecule which, in addition to inhibiting trypsin and **plasmin** activities directly, is able toblock unoccupied uPAR, thereby preventing localization

of **uPA** activity.

L15 ANSWER 11 OF 17 MEDLINE on STN DUPLICATE 8
96188781. PubMed ID: 8604004. Endogenously produced urokinase amplifies tumor necrosis factor-alpha secretion by THP-1 mononuclear phagocytes. Sitrin R G; Shollenberger S B; Strieter R M; Gyetko M R. (Department of Internal Medicine, University of Michigan, Ann Arbor 48109-0360, USA.) Journal of leukocyte biology, (1996 Feb) 59 (2) 302-11. Journal code: 8405628. ISSN: 0741-5400. Pub. country: United States. Language: English.
AB This study examined the effects of endogenous urokinase (**uPA**) on lipopolysaccharide (LPS)-stimulated tumor necrosis factor alpha (TNF-alpha) secretion in THP-1 mononuclear phagocytes. Anti-**uPA** monoclonal antibody (mAb) suppressed LPS-driven TNF-alpha secretion by 61.6 +/- 5.9% (P<.001), and PAI-1, a **uPA** inhibitor, suppressed it to 53.1 +/- 8.2% of the control value (P<.001). Up-regulation of TNF-alpha mRNA was suppressed in parallel with secreted TNF-alpha protein. TNF-alpha secretion was unaffected by depleting **plasminogen** or by aprotinin, a **plasmin** inhibitor. When endogenous **uPA** was displaced from the cell, exogenous high-molecular-**weight** (intact) **uPA** augmented LPS-driven TNF-alpha secretion. By contrast, a **uPA fragment** containing the catalytic domain was inhibitory, and the **uPA** receptor-binding domain had no effect. We conclude that endogenous **uPA** amplifies TNF-alpha neosynthesis of LPS-stimulated THP-1 mononuclear phagocytes. The effect requires intact **uPA** and is independent of **plasmin** activity. This represents a novel mechanism by which a mononuclear phagocyte-derived protease contributes to generating proinflammatory signals.

L15 ANSWER 12 OF 17 MEDLINE on STN DUPLICATE 9
94149075. PubMed ID: 8106568. Regulation of in vitro glia-induced microvessel morphogenesis by urokinase. Laterra J; Indurti R R; Goldstein G W. (Department of Neurology, Kennedy Krieger Research Institute, Johns Hopkins Medical Institutions, Baltimore, Maryland 21205.) Journal of cellular physiology, (1994 Feb) 158 (2) 317-24. Journal code: 0050222. ISSN: 0021-9541. Pub. country: United States. Language: English.
AB **Plasminogen** activators (PAs) regulate a variety of processes involved in tissue morphogenesis and differentiation. We used a coculture system in which microvascular endothelial cells are induced by glial cells to form capillary-like structures in order to examine the role of urokinase-type PA (**uPA**) during microvessel morphogenesis within the central nervous system (CNS). Endothelia-derived **uPA** activity decreased sevenfold within glial-endothelial cocultures when capillary-like structures were formed. Incubation of cocultures with concentrations of phorbol 12-myristate 13-acetate (0.1 and 1.0 nM) that induced endothelial **uPA** activity (45-210%) inhibited endothelial differentiation (25-70%). Furthermore, incubation of cocultures with proteolytically active low molecular **weight uPA** (5-500 IU/ml) inhibited endothelial differentiation (37-75%), whereas the amino terminal cell-binding **fragment** of **uPA** had minimal effect. Inhibition of **plasminogen** activation in cocultures with the serine protease/**plasmin** inhibitors aprotinin and soybean trypsin inhibitor increased glia-induced capillary-like structure formation (96-98%). These findings establish a paracrine/autocrine function for urokinase and its inhibitors in regulating endothelial responses to perivascular glia and provide insight into mechanisms of microvascular reactions to CNS pathology.

L15 ANSWER 13 OF 17 MEDLINE on STN DUPLICATE 10
92198395. PubMed ID: 1801751. Biological and clinical relevance of the urokinase-type **plasminogen** activator (**uPA**) in breast cancer. Schmitt M; Goretzki L; Janicke F; Calvete J; Eulitz M; Kobayashi H; Chucholowski N; Graeff H. (Frauenklinik, Technischen Universitat Munchen, Klinikum rechts der Isar, FRG.) Biomedica biochimica acta, (1991) 50 (4-6) 731-41. Journal code: 8304435. ISSN: 0232-766X. Pub.

country: GERMANY: Germany, Federal Republic of. Language: English.

AB Tumor cell invasion and metastasis is a multifactorial process, which at each step may require the action of proteolytic enzymes such as collagenases, cathepsins, **plasmin**, or **plasminogen** activators. An enzymatically inactive proenzyme form of the urokinase-type **plasminogen** activator (pro-**uPA**) is secreted by tumor cells which may be converted to an enzymatically active two-chain **uPA**-molecule (HMW-**uPA**) by **plasmin** -like enzymes. Action of proteases on pro-**uPA** may generate the enzymatically active or inactive high-molecular-**weight** form of **uPA** (HMW-**uPA**). Some proteases (**plasmin**, cathepsin B and L, kallikrein, trypsin or thermolysin) activate pro-**uPA** by cleaving the peptide bond Lys158 and IIe159. Other proteases (elastase, thrombin) cleave pro-**uPA** at different positions to yield enzymatically inactive HMW-**uPA.** HMW-**uPA** may be split into the enzymatically active LMW-**uPA** and the enzymatically inactive ATF (amino terminal **fragment**). ATF may be cleaved between peptide sequence 20 and 40 within the receptor binding domain of **uPA** (GFD). Such impaired ATF does not bind to **uPA**-receptors. Action of the bacterial endoproteinase Asp-N from Pseudomonas fragi mutant on pro-**uPA** or HMW-**uPA**, however, generates intact ATF which efficiently competes for binding of HMW-**uPA** or pro-**uPA** to receptors on tumor cells. High **uPA**-antigen content (pro-**uPA**, HMW-**uPA**, or LMW-**uPA**) in breast cancer tissue (not in plasma) indicates an elevated risk for the patient of recurrences and shorter overall survival. Thus pro-**uPA**/**uPA**-antigen content in breast cancer tissue serves as an independent prognostic parameter for the outcome of the disease. Cathepsin D is also an independent prognostic factor for recurrences and overall survival. High content of cathepsin D in breast cancer tumors is, however, not correlated with elevated levels of pro-**uPA**/**uPA** indicating that synthesis and release of cathepsin D and pro-**uPA**/**uPA** are independent events.

L15 ANSWER 14 OF 17 CAPLUS COPYRIGHT 2005 ACS on STN

1989:548038 Document No. 111:148038 Method for preparing single chain urokinase. Cassani, Giovanni; Robbiati, Federico Maria; Blasi, Francesco; Nolli, Marialuisa; Corti, Angelo (Gruppo Lepetit S.p.A., Italy). Eur. Pat. Appl. EP 303028 A1 19890215, 54 pp. DESIGNATED STATES: R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1988-109800 19880620. PRIORITY: GB 1987-18877 19870810; US 1988-173337 19880324.

AB The human urokinase gene is cloned and expressed in tissue culture cells using animal virus-derived expression vectors. The promoter region of the gene is used in expression vectors. Mammalian cells, e.g. CHO, murine L-WG, were transformed with a number of Rous Sarcoma virus expression vectors containing the urokinase gene and the protein was purified from the culture medium by immune-affinity chromatog. When the proteins were separated by SDS-PAGE, Western blots showed the proteins to be mainly in the single-chain form with some variability in the mol. **weight** This was shown to be due to variable glycosylation by loss of heterogeneity upon treatment with carbohydrases. These proteins also reacted with three monoclonal antibodies and had the correct N- and C-termini. The proteins were cleaved accurately by hum **plasmin** to give an enzyme with similar kinetic properties to the urinary enzyme. The recombinant enzyme was normally inhibited by **plasminogen** activator inhibitor. The promoter region was isolated and studied using deletion derivs. and a reporter gene to find a more efficient promoter.

L15 ANSWER 15 OF 17 MEDLINE on STN

89296897. PubMed ID: 2544876. Accessibility of receptor-bound urokinase to type-1 **plasminogen** activator inhibitor. Cubellis M V; Andreasen P; Ragno P; Mayer M; Dano K; Blasi F. (Institute of Microbiology, University of Copenhagen, Denmark.) Proceedings of the National Academy of Sciences of the United States of America, (1989 Jul) 86 (13) 4828-32.

Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Urokinase **plasminogen** activator (**uPA**) interacts with a surface receptor and with specific inhibitors, such as **plasminogen** activator inhibitor type 1 (PAI-1). These interactions are mediated by two functionally independent domains of the molecule: the catalytic domain (at the carboxyl terminus) and the growth factor domain (at the amino terminus). We have now investigated whether PAI-1 can bind and inhibit receptor-bound **uPA.** Binding of 125I-labeled ATF (amino-terminal **fragment** of **uPA**) to human U937 monocyte-like cells can be competed for by **uPA**-PAI-1 complexes, but not by PAI-1 alone. Performed 125I-labeled **uPA**-PAI-1 complexes can bind to **uPA** receptor with the same binding specificity as **uPA.** PAI-1 also binds to, and inhibits the activity of, receptor-bound **uPA** in U937 cells, as shown in U937 cells by a caseinolytic plaque assay. **Plasminogen** activator activity of these cells is dependent on exogenous **uPA**, is competed for by receptor-binding diisopropyl fluorophosphate-treated **uPA**, and is inhibited by the addition of PAI-1. In conclusion, in U937 cells the binding to the receptor does not shield **uPA** from the action of PAI-1. The possibility that in adherent cells a different localization of PAI-1 and **uPA** leads to protection of **uPA** from PAI-1 is to be considered.

L15 ANSWER 16 OF 17 MEDLINE on STN

88135650. PubMed ID: 2963689. Modulation of metastatic potential by cell surface urokinase of murine melanoma cells. Hearing V J; Law L W; Corti A; Appella E; Blasi F. (Laboratory of Cell Biology, National Cancer Institute, NIH, Bethesda, Maryland 20892.) Cancer research, (1988 Mar 1) 48 (5) 1270-8. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB We have carried out enzymatic, immunofluorescence, and surface iodination studies which show that B16 melanoma cells express the single chain form of the urokinase type **plasminogen** activator (**uPA**) on their cell surface, and that these cells are capable of **plasminogen**-dependent fibronectin degradation. The significance of the expression of surface single-chain **uPA** and **uPA** activity to the metastatic process was examined by preincubating melanoma cells with **uPA** modulating agents followed by i.v. injection of the cells into mice and enumeration of pulmonary nodules 17 days later. B16 cells that had been pretreated with anti-**uPA** immunoglobulins that were inhibitory to **uPA** activity invariably showed significantly decreased numbers of metastases compared to controls. On the contrary, pretreatment with **plasmin**, which is not only the product of the **uPA** catalyzed reaction but is also able to convert single-chain **uPA** to **uPA**, significantly increased the numbers of metastases. Control treatments, which included normal rabbit and mouse immunoglobulins, monovalent noninhibitory anti-**uPA** Fab **fragments**, and various monoclonal and polyclonal antibodies directed against other B16 cell surface antigens, did not affect the metastatic potential of the cells. Divalent inhibitory anti-**uPA** F(ab)2 **fragments**, on the contrary, inhibited metastasis as efficiently as intact IgG. The results support the hypothesis that proteolysis of extracellular matrix components by cell surface-localized **uPA** may be a critical step during the process of tumor cell invasion and metastasis.

L15 ANSWER 17 OF 17 MEDLINE on STN DUPLICATE 11

87275923. PubMed ID: 3609016. Effect of the cyanogen-bromide-2 **fragment** of fibrinogen on **plasminogen** activation by single-chain urokinase-type **plasminogen** activator. Kirchheimer J C; Resch I; Christ G; Wojta J; Binder B R. European journal of biochemistry / FEBS, (1987 Jul 15) 166 (2) 393-7. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB Activation of Glu-**plasminogen** by single-chain urokinase-type **plasminogen** activator (sc-**uPA**), isolated from human urine, was studied in a purified system in the absence and presence of the cyanogen bromide fibrinogen **fragment**, FCB 2, and compared to **plasminogen** activation by two-chain high-Mr urokinase. **Plasminogen** activation by sc-**uPA** was significantly increased by the FCB-2 fibrinogen **fragment**, an effect brought about by decrease of apparent Km and increase of apparent kcat. During the course of **plasminogen** activation by scu-PA, two-chain urokinase was formed from 125I-sc-**uPA** to a significant degree only when a concentration of 30 nM **plasmin** was reached in the incubation mixture; this was only the case in the system stimulated by FCB-2 fibrinogen **fragment** and only after 30 min. Formation of two-chain urokinase was not, however, related to the increase in the rate of **plasmin** formation induced by the FCB-2 fibrinogen **fragment.**

=> s angiostatin
L16 3849 ANGIOSTATIN

=> s l16 and molecular weight
L17 86 L16 AND MOLECULAR WEIGHT

=> s l17 and 34
L18 0 L17 AND 34

=> s l17 and plasminogen
L19 58 L17 AND PLASMINOGEN

=> dup remove l19
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L20 33 DUP REMOVE L19 (25 DUPLICATES REMOVED)

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L20 ANSWER 1 OF 33 MEDLINE on STN DUPLICATE 1
2005187782. PubMed ID: 15713679. Biochemical and enzymatic characterization of human kallikrein 5 (hK5), a novel serine protease potentially involved in cancer progression. Michael Iacovos P; Sotiropoulou Georgia; Pampalakis Georgios; Magklara Angeliki; Ghosh Manik; Wasney Greg; Diamandis Eleftherios P. (Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada.) Journal of biological chemistry, (2005 Apr 15) 280 (15) 14628-35. Electronic Publication: 2005-02-15. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Human kallikrein 5 (KLK5) is a member of the human kallikrein gene family of serine proteases. Preliminary results indicate that the protein, hK5, may be a potential serological marker for breast and ovarian cancer. Other studies implicate hK5 with skin desquamation and skin diseases. To gain further insights on hK5 physiological functions, we studied its substrate specificity, the regulation of its activity by various inhibitors, and identified candidate physiological substrates. After producing and purifying recombinant hK5 in yeast, we determined the k(cat)/K(m) ratio of the fluorogenic substrates Gly-Pro-Arg-AMC and Gly-Pro-Lys-AMC, and showed that it has trypsin-like activity with strong preference for Arg over Lys in the P1 position. The serpins alpha(2)-antiplasmin and antithrombin were able to inhibit hK5 with an inhibition constant (k(+2)/K(i)) of 1.0 x 10(-) (2)and 4.2 x 10(-4) m(-1) min(-1), respectively. No inhibition was observed with the serpins alpha(1)-antitrypsin and alpha(1)-antichymotrypsin, although alpha(2)-macroglobulin partially inhibited hK5 at high concentrations. We also demonstrated that hK5 can efficiently digest the extracellular matrix components, collagens type I, II, III, and IV, fibronectin, and laminin. Furthermore, our results suggest that hK5 can potentially release (a)

angiostatin 4.5 from **plasminogen**, (b) "cystatin-like domain 3" from low **molecular weight** kininogen, and (c) fibrinopeptide B and peptide beta15-42 from the Bbeta chain of fibrinogen. hK5 could also play a role in the regulation of the binding of **plasminogen** activator inhibitor 1 to vitronectin. Our findings suggest that hK5 may be implicated in tumor progression, particularly in invasion and angiogenesis, and may represent a novel therapeutic target.

L20 ANSWER 2 OF 33 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2005291144 EMBASE Functional characterization of neostatins, the MMP-derived, enzymatic cleavage products of type XVIII collagen. Chang J.-H.; Javier J.A.D.; Chang G.-Y.; Oliveira H.B.; Azar D.T.. D.T. Azar, Corneal, External Disease and Refractive Surgery Service, Massachusetts Eye and Ear Infirmary, Harvard Medical School, 243 Charles Street, Boston, MA 02114, United States. dazar@meei.harvard.edu. FEBS Letters Vol. 579, No. 17, pp. 3601-3606 27 Jul 2005.
Refs: 40.
ISSN: 0014-5793. CODEN: FEBLAL
S 0014-5793(05)00649-6. Pub. Country: Netherlands. Language: English. Summary Language: English.

ED Entered STN: 20050818

AB Several anti-angiogenic factors are derived from proteolytic processing of large molecules including endostatin from type XVIII collagen and **angiostatin** from **plasminogen.** In previous studies we showed that neostatin-7, the C-terminal 28 kDa endostatin-spanning proteolytic fragment, is generated from the proteolytic action of matrix metalloproteinase matrilysin (MMP)-7 on type XVIII collagen. Now, we report a second member of the neostatin family of proteins, neostatin-14. Given the small quantities of neostatin-7 and -14 generated by the breakdown of naturally occurring collagen XVIII (using MMP-7 and -14, respectively), we used two other approaches to characterize the anti-angiogenic properties of these molecules: murine recombinant neostatin in vitro, and gene therapy. We demonstrate that murine recombinant neostatin-7 inhibits calf pulmonary artery endothelial cell proliferation and that microinjection of neostatin-7 and neostatin-14 naked DNA into the corneal stroma of mice results in significant reduction of basic fibroblast growth factor-induced corneal neovascularization. These results provide supportive evidence of the possible anti-angiogenic effect of neostatins. .COPYRGT. 2005 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

L20 ANSWER 3 OF 33 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2005:765640 The Genuine Article (R) Number: 945VH. Angiogenesis inhibitors found within the haemostasis pathway. Staton C A; Lewis C E (Reprint). Univ Sheffield, Sch Med, Tumor Targeting Grp, Sheffield S10 2RX, S Yorkshire, England (Reprint); Univ Sheffield, Sch Med, Microcirculat Res Grp, Sheffield, S Yorkshire, England. Claire.lewis@sheffield.ac.uk. JOURNAL OF CELLULAR AND MOLECULAR MEDICINE (APR-JUN 2005) Vol. 9, No. 2, pp. 286-302. ISSN: 1582-1838. Publisher: CAROL DAVILA UNIV PRESS, 8 EROILOR SANITARI BLVD, BUCHARESST 76241, ROMANIA. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Angiogenesis, the development of new blood vessels from the existing vasculature, and haemostasis, the coagulation cascade leading to formation of a clot, are among the most consistent host responses associated with cancer. Importantly, these two pathways interrelate, with blood coagulation and fibrinolysis influencing tumor angiogenesis directly, thereby contributing to tumor growth. Moreover, many endogenous inhibitors of angiogenesis are found within platelets or harboured as cryptic fragments of haemostatic proteins. In this review we outline ways in which angiogenesis is coordinated and regulated by haemostasis in human cancer. Then we detail the experimental and preclinical evidence for the ability of many of these endogenous proteins to inhibit tumor angiogenesis and thus their potential to be anti-cancer agents, with particular

reference to any clinical trials.

L20 ANSWER 4 OF 33 MEDLINE on STN DUPLICATE 2
2005196581. PubMed ID: 15828939. Multiple fragments related to **angiostatin** and endostatin in fluid from venous leg ulcers. Smith Ewen; Hoffman Richard. (Department of Biosciences, University of Hertfordshire, Hatfield, Hertfordshire, United Kingdom.) Wound repair and regeneration : official publication of the Wound Healing Society [and] European Tissue Repair Society, (2005 Mar-Apr) 13 (2) 148-57. Journal code: 9310939. ISSN: 1067-1927. Pub. country: United States. Language: English.

AB To investigate whether compromised angiogenesis could contribute to the impaired healing of venous leg ulcers, we have analyzed fluids from venous leg ulcers for the presence of the angiogenesis inhibitors **angiostatin** and endostatin. Multiple fragments related to **angiostatin** were detected by Western blot analysis. One **angiostatin** fragment was identified by mass spectrometry as **plasminogen** kringle domains 1-3 containing amino acids 82-343 of **plasminogen**, and a fraction containing this fragment inhibited tubule formation of human umbilical vein endothelial cells in a Matrigel assay. The leg ulcer fluids also contained endogenous endostatin (20 kDa) as well as higher **molecular weight** endostatin-related proteins. The concentrations of endostatin in the wound fluids, which ranged from 12.8 to 65.5 ng/ml, were higher than the concentration in human serum (7.7 ng/ml). Most of the endostatin in leg ulcer fluid appeared to be bound to the proteoglycan glypican-1. These data suggest that anti-angiogenic activity is present at the site of venous leg ulcers, and at least in the case of **angiostatin**, is biologically active.

L20 ANSWER 5 OF 33 MEDLINE on STN DUPLICATE 3
2005101833. PubMed ID: 15711718. Integrin-mediated leukocyte adhesive interactions: regulation by haemostatic factors. Chavakis T; Preissner K T. (Medizinische Klinik I, Universitatsklinikum Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany.. triantafyllos.chavakis@med.uni-heidelberg.de) . Hamostaseologie, (2005 Feb) 25 (1) 33-8. Ref: 68. Journal code: 8204531. ISSN: 0720-9355. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB Leukocyte recruitment to sites of inflammation, infection or vascular injury is a complex event that is orchestrated by a tightly coordinated sequence of interactions between leukocytes and cells of the vessel wall, especially endothelial cells. These interactions are controlled by the expression and activation of various adhesion receptors and protease systems. This review will focus on novel aspects of the regulation of integrin-dependent leukocyte adhesion by haemostatic factors. Here, so-called non-haemostatic properties of endogenous proteins such as high **molecular weight** kininogen, urokinase receptor, urokinase, as well as **plasminogen** and its cleavage product **angiostatin** in leukocyte adhesion and transmigration will be summarized. The crosstalk between haemostatic factors and inflammatory reactions may contribute to a better understanding of inflammatory vascular disorders and to the development of novel therapeutical concepts.

L20 ANSWER 6 OF 33 EMBASE on STN
2004093505 EMBASE Nonlysine-analog plasmmogen modulators promote autoproteolytic generation of plasmin(ogen) fragments with **angiostatin**-like activity. Ohyama S.; Harada T.; Chikanishi T.; Miura Y.; Hasumi K.. K. Hasumi, Dept. of Applied Biological Science, Tokyo Noko University, 3-5-8 Saiwaicho, Fuchu-shi, Tokyo, 183-8509, Japan. hasumi@cc.tuat.ac.jp. European Journal of Biochemistry Vol. 271, No. 4, pp. 809-820 2004.
Refs: 41.
ISSN: 0014-2956. CODEN: EJBCAI
Pub. Country: United Kingdom. Language: English. Summary Language: English.

ED Entered STN: 20040325
AB We recently discovered several nonlysine-analog conformational modulators for **plasminogen.** These include SMTP-6, thioplabin B and complestatin that are low molecular mass compounds of microbial origin. Unlike lysine-analog modulators, which increase **plasminogen** activation but inhibit its binding to fibrin, the nonlysine-analog modulators enhance both activation and fibrin binding of **plasminogen.** Here we show that some nonlysine-analog modulators promote autoproteolytic generation of plasmin(ogen) derivatives with its catalytic domain undergoing extensive fragmentation (PMDs), which have **angiostatin**-like anti-endothelial activity. The enhancement of urokinase-catalyzed **plasminogen** activation by SMTP-6 was followed by rapid inactivation of plasmin due to its degradation mainly in the catalytic domain, yielding PMD with a molecular mass ranging from 68 to 77 kDa. PMD generation was observed when plasmin alone was treated with SMTP-6 and was inhibited by the plasmin inhibitor aprotinin, indicating an autoproteolytic mechanism in PMD generation. Thioplabin B and complestatin, two other nonlysine-analog modulators, were also active in producing similar PMDs, whereas the lysine analog 6-aminohexanoic acid was inactive while it enhanced **plasminogen** activation. Peptide sequencing and mass spectrometric analyses suggested that plasmin fragmentation was due to cleavage at Lys615-Val616, Lys651-Leu652, Lys661-Val662, Lys698-Glu699, Lys708-Val709 and several other sites mostly in the catalytic domain. PMD was inhibitory to proliferation, migration and tube formation of endothelial cells at concentrations of 0.3-10 μg.ovrhdot.mL (-1). These results suggest a possible application of nonlysine-analog modulators in the treatment of cancer through the enhancement of endogenous plasmin(ogen) fragment formation.

L20 ANSWER 7 OF 33 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2004:771076 The Genuine Article (R) Number: 847VT. Histidine-Proline Rich Glycoprotein (HPRG) binds and transiduces anti-angiogenic signals through cell surface tropomyosin on endothelial cells. Guan X J; Juarez J C; Qi X P; Shipulina N V; Shaw D E; Morgan W T; McCrae K R; Mazar A R; Donate F (Reprint). Attenuon LLC, 10130 Sorrento Vallety Rd, Suite B, San Diego, CA 92121 USA (Reprint); Attenuon LLC, San Diego, CA 92121 USA; Univ Missouri, Div Mol Biol & Biochem, Sch Biol Sci, Kansas City, KS USA; Case Western Reserve Univ, Sch Med, Div Hematol Oncol, Cleveland, OH USA; DE Shaw Res & Dev LLC, New York, NY USA. donate@attenuon.com. THROMBOSIS AND HAEMOSTASIS (AUG 2004) Vol. 92, No. 2, pp. 403-412. ISSN: 0340-6245. Publisher: SCHATTAUER GMBH-VERLAG MEDIZIN NATURWISSENSCHAFTEN, HOLDERLINSTRASSE 3, D-70174 STUTTGART, GERMANY. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The anti-angiogenic properties of the histidine-proline-rich (H/P) domain of HPRG have recently been described (Juarez JC, et al. Cancer Research 2002; 62: 5344-50). However, the binding site that mediates these properties is unknown. HPRG is evolutionarily, functionally and structurally related to cleaved high **molecular weight** kininogen (HKa), an anti-angiogenic polypeptide that stimulates apoptosis of proliferating endothelial cells through binding to cell-surface tropomyosin (Zhang J-C, et al. Proc Natl Acad Sci USA 2002; 99: 12224-9). In this study, we demonstrate that HPRG binds with high affinity to FGF-2-stimulated human umbilical vein endothelial cells (HUVEC) and immobilized tropomyosin in a Zn2+ or pH-dependent manner, and that this interaction is mediated by the H/P domain of HPRG. At least two binding sites for HPRG, tropomyosin and heparan sulfate proteoglycans (HSPs), were identified on the surface of FGF-2-activated endothelial cells. Translocation of tropomyosin to the surface of HUVEC occurred in response to FGF-2, and the anti-angiogenic activity of HPRG in a Matrigel plug model was partially inhibited by soluble tropomyosin. These results suggest that HPRG binds to endothelial cell surface tropomyosin which at least partially mediates the anti-angiogenic effects of HPRG.

L20 ANSWER 8 OF 33 EMBASE

on STN DUPLICATE 4

2004178495 EMBASE Fibrinogen catabolism within the procoagulant VX-2 tumor of rabbit lung in vivo: Effluxing fibrin(ogen) fragments contain antiangiogenic activity. Hatton M.W.C.; Southward S.M.R.; Legault K.J.; Ross B.L.; Clarke B.J.; Bajzar L.; Blajchman M.A.; Singh G.; Richardson M.. Dr. M.W.C. Hatton, Dept. of Pathol. and Molec. Medicine, McMaster Univ. Hlth. Sciences Centre, 1200 Main St W, Hamilton, Ont. L8N 3Z5, Canada. hattonm@mcmaster.ca. Journal of Laboratory and Clinical Medicine Vol. 143, No. 4, pp. 241-254 2004.
Refs: 41.
ISSN: 0022-2143. CODEN: JLCMAK
Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 20040513
AB Many types of solid tumors are known to be procoagulant environments. This is partly because a hyperpermeable vascular system within the tumor allows plasma hemostatic factors to accumulate in relatively high concentrations in the stroma, and many solid-tumor cells express tissue factor or a procoagulant factor. These circumstances appear to exist in the VX-2 lung tumor of the New Zealand White (NZW) rabbit, and they sustain a measurable turnover of stromal deposits of fibrin(ogen). We have measured the turnover of fibrinogen within tumors of the VX-2 tumor-burdened rabbit and analysed the catabolic products of fibrin(ogen) and the status of fibrinolysis in tumor-derived interpleural effusate. Using intravenously injected (125)I-labeled rabbit fibrinogen as a marker, we found that fibrinogen (approximate blood concentration 1740 μg/mL) passed from blood to VX-2 tumor stroma, saturating the tumor at a concentration of approximately 348 μg fibrinogen/g in approximately 12 hours. We measured fibrin(ogen) fragments, at a concentration of approximately 292 μg/ml, in interpleural effusates that we recovered from 13% of the VX-2-burdened rabbits. Unreduced fibrin(ogen) fragments consisted of 4 major components with a relative molecular mass of approximately 250,000 (assumed to be fragment X; approximately 9% of total fragments from densitometry of immunoblots), 200,000 (D-dimer; 41%), 110,000 (fragment D; 49%), and 50,000 to 55,000 (fragment E; 1%-2%) kD. Total fibrin(ogen) fragments immunopurified from effusates exhibited an antiangiogenic effect when subjected to a chick embryo chorioallantoic membrane procedure. Interpleural effusates were devoid of plasmin activity or active **plasminogen** activator inhibitor-1 but contained plasmin complexes and active urokinase-like **plasminogen** activator (uPA), α(2)-antiplasmin, and thrombin-activatable fibrinolysis inhibitor. We speculate that VX-2 cells release uPA to activate fibrinolysis within the tumor stroma. Catabolic products of hemostasis (eg, fibrinolytic fragments, **angiostatin**) flux from the stroma into the interpleural space, thereby providing a net antiangiogenic property to the effusate and ultimately to the lymphatic and circulatory systems.

L20 ANSWER 9 OF 33 EMBASE
on STN

2004054711 EMBASE **Plasminogen** fragmentation and increased production of extracellular matrix-degrading proteinases are associated with serous epithelial ovarian cancer progression. Murthi P.; Barker G.; Nowell C.J.; Rice G.E.; Baker M.S.; Kalionis B.; Quinn M.A.. P. Murthi, Dept. of Obstetrics and Gynaecology, Royal Women's Hospital, University of Melbourne, 132, Grattan Street, Carlton, Vic. 3053, Australia. padma@unimelb.edu.au. Gynecologic Oncology Vol. 92, No. 1, pp. 80-88 2004.
Refs: 37.
ISSN: 0090-8258. CODEN: GYNOA3
Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 20040220
AB Objective. Elevated levels of proteases are linked to the malignant phenotype in a wide variety of solid tumors. Therefore, the expression of **plasminogen**, matrix metalloproteinases (MMP-2 and MMP-9), and of the serine protease urokinase-type **plasminogen** activator (uPA)

in serous epithelial carcinoma of the ovary were investigated. Methods. **Plasminogen** antigen was analyzed in tissue extracts and in the urine of patients with normal (n = 12), benign (n = 6), borderline (n = 9), and invasive serous tumors (n = 22) by Western immunoblotting using rabbit polyclonal **plasminogen** and murine monoclonal **angiostatin** antibodies. In the same tissue extracts, semiquantitative estimates of MMP-2, MMP-9, total MMP activity, and uPA activity were determined using semiquantitative gelatin zymography in the presence or absence of human **plasminogen.** Results. Bands corresponding to Glu-**plasminogen** (approximately 92 kDa) and Lys-**plasminogen** (approximately 86 kDa) were detected in all ovarian tissues and in corresponding urine samples. Densitometric analysis of combined Glu- or Lys-**plasminogen** levels showed significantly decreased levels in malignant compared to normal tissue. In Grade 3 cancers, there was no evidence of Glu-**plasminogen** or **angiostatin.** MMP activity was significantly elevated in both borderline and in Grade 3 ovarian cancer tissues. Increased tissue uPA activity on zymograms was detected only in Grade 3 ovarian cancer tissue. Conclusion. These data suggest that proteolytic activity of the **plasminogen** activation cascade increases in serous epithelial ovarian carcinoma.

L20 ANSWER 10 OF 33 MEDLINE on STN

2004279470. PubMed ID: 15177278. Characterization and biological activities of recombinant human **plasminogen** kringle 1-3 produced in Escherichia coli. You Weon-Kyoo; So Seung-Ho; Sohn Young-Doug; Lee Hyosil; Park Doo-Hong; Chung Soo-Il; Chung Kwang-Hoe. (Mogam Biotechnology Research Institute, Bioproducts Research Center, 341 Pojung-ri, Koosung-myun, Yongin City, Kyonggi-do 449-910, Republic of Korea.) Protein expression and purification, (2004 Jul) 36 (1) 1-10. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB Angiogenesis, the formation of new capillaries from preexisting blood vessels, is involved in many pathological conditions, for example, tumorigenesis, diabetic retinopathy, and rheumatoid arthritis. **Angiostatin**, which contains the kringle 1-4 domains of **plasminogen**, is known to be a potent inhibitor of angiogenesis and a strong suppressor of various solid tumors. In this study, we expressed recombinant protein containing the kringle 1-3 domains of human **plasminogen** in Escherichia coli and investigated its biological activities. The protein was successfully refolded from inclusion bodies and purified at a 30% overall yield, as a single peak by HPLC. The purified recombinant protein had biochemical properties that were similar to those of the native form, which included molecular size, lysine-binding capacity, and immunoreactivity with a specific antibody. The recombinant protein was also found to strongly inhibit the proliferation of bovine capillary endothelial cells in vitro, and the formation of new capillaries on chick embryos. In addition, it suppressed the growth of primary Lewis lung carcinoma and B16 melanoma in an in vivo mouse model. Our findings suggest that the recombinant kringle 1-3 domains in a prokaryote expression system have anti-angiogenic activities, which may be useful in clinical and basic research in the field of angiogenesis.

L20 ANSWER 11 OF 33 EMBASE on STN

2004177596 EMBASE **Angiostatin** and matrix metalloprotease expression following ischemic acute renal failure. Basile D.P.; Fredrich K.; Weihrauch D.; Hattan N.; Chilian W.M.. D.P. Basile, Dept. of Physiology, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226, United States. dbasile@mcw.edu. American Journal of Physiology - Renal Physiology Vol. 286, No. 5 55-5, pp. F893-F902 2004.
Refs: 51.
ISSN: 0363-6127. CODEN: AJPPFK
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 20040513

AB Ischemic injury to the kidney results in blood vessel loss and predisposition to chronic renal disease. **Angiostatin** is a proteolytic cleavage product of **plasminogen** that inhibits angiogenesis, promotes apoptosis of endothelial cells, and disrupts capillary integrity. A combination of lysine-Sepharose enrichment followed by Western blotting was used to study the expression of **angiostatin** in response to the induction of ischemic renal injury. No **angiostatin** products were readily detectable in kidneys of sham-operated control rats. In contrast, both 38- and 50-kDa forms of **angiostatin** were dramatically enhanced in the first 3 days following 45-min ischemia-reperfusion injury. Renal **angiostatin** levels declined but remained detectable at late time points postrecovery (8-35 days postischemia). **Angiostatin**-like immunoreactivity was also elevated in the plasma and in urine for up to 35 days following injury. Lysine-Sepharose extracts of either kidney or urine inhibited vascular endothelial cell growth factor-induced proliferation of human aortic endothelial cells in vitro; an effect that was blocked by coincubation with an **angiostatin** antibody. RT-PCR verified that mRNA of the parent protein **plasminogen** was produced in the liver, but it was not present in either sham-operated or postischemic kidney. Matrix metalloproteinase (MMP)-2 and MMP-9, which may mediate **angiostatin** generation, were enhanced in postischemic kidney tissue and were localized to the renal tubules, interstitial cells, and the tubulo-interstitial space. These data indicate the possible local synthesis of **angiostatin** following acute renal failure (ARF) and suggest a possible role for MMPs in this activity. Renal **angiostatin** generation following ARF may modulate renal capillary density postischemia and thereby influence chronic renal function.

L20 ANSWER 12 OF 33 CAPLUS COPYRIGHT 2005 ACS on STN
2003:232736 Document No. 138:251103 **Plasminogen** fragments possessing angiogenesis inhibitory activity, and method for screening chemical compound capable of inducing formation of **plasminogen** fragments. Hasumi, Keiji; Oyama, Shigeki; Harada, Tomotaka; Hu, Wei Min (TTC K. K., Japan). Jpn. Kokai Tokkyo Koho JP 2003088397 A2 20030325, 12 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 2001-328411 20010919.
AB An efficient method is provided for screening a chemical compound which promotes the conversion of **plasminogen** to its fragment possessing the **angiostatin**-like activity using as an index the promotion of plamingen activation. Also provided is the **plasminogen** fragments generated by the function of the chemical compound identified by this method. These **plasminogen** fragments are novel mols., which possesses the **mol. weight** in the range of 50-72kda and contains K1-K5, and in which a portion of the protease region is linked with a disulfide bond. These mols. inhibit the proliferation, migration and angiogenesis of cultured vascular endothelium cells like **angiostatin.**

L20 ANSWER 13 OF 33 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2003503768 EMBASE Hemostatic regulators of tumor angiogenesis: A source of antiangiogenic agents for cancer treatment?. Daly M.E.; Makris A.; Reed M.; Lewis C.E.. Dr. C.E. Lewis, Academic Unit of Pathology, Sch. of Medicine/Biomedical Sciences, Beech Hill Rd., Sheffield S10 2RX, United Kingdom. Claire.lewis@sheffield.ac.uk. Journal of the National Cancer Institute Vol. 95, No. 22, pp. 1660-1673 19 Nov 2003.
Refs: 167.
ISSN: 0027-8874. CODEN: JNCIAM
Pub. Country: United Kingdom. Language: English. Summary Language: English.
ED Entered STN: 20031230
AB The maintenance of vascular integrity and control of blood loss are regulated by a sophisticated system of circulating and cell-associated hemostatic factors. These factors control local platelet aggregation, the conversion of soluble fibrinogen to an insoluble fibrin polymer, and the

dissolution of fibrin. However, hemostatic factors are also involved in a number of physiologic processes, including development, tissue remodeling, wound repair, reproduction, inflammation, and angiogenesis. In this review, we outline ways in which angiogenesis is coordinated with and regulated by hemostasis. We focus on inhibitors of angiogenesis contained within platelets or harbored as cryptic fragments of hemostatic proteins and assess the experimental and preclinical evidence for their ability to inhibit tumor angiogenesis and, thus, their potential to be anticancer agents. Finally, we review the results of recent clinical trials involving angiogenesis inhibitors and the evidence that antiangiogenic therapy may be associated with hemostatic complications.

L20 ANSWER 14 OF 33 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2003:503260 Document No.: PREV200300498838. NG2 expression is lost during melanoma progression. Westphal, Johan R. [Reprint Author]; van Kraats, Annemieke; de Groot-Besseling, Renate; Ruers, Theo; Ruiter, Dirk; de Waal, Rob. Dept. Pathology, University Medical Center Nijmegen, Nijmegen, Netherlands. Proceedings of the American Association for Cancer Research Annual Meeting, (July 2003) Vol. 44, pp. 1250. print.
Meeting Info.: 94th Annual Meeting of the American Association for Cancer Research. Washington, DC, USA. July 11-14, 2003.
ISSN: 0197-016X. Language: English.

L20 ANSWER 15 OF 33 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2003:381626 Document No.: PREV200300381626. Benzamidine binding to **plasminogen** changes availability of cleavage sites for human neutrophil elastase. Warejcka, Debra J. [Reprint Author]; Twining, Sally S.. Biochemistry, Medical College of Wisconsin, 8701 W. Watertown Plank Rd., Milwaukee, WI, 53226, USA. dwarejck@mcw.edu; stwining@mcw.edu. FASEB Journal, (March 2003) Vol. 17, No. 4-5, pp. Abstract No. 633.7. http://www.fasebj.org/. e-file.
Meeting Info.: FASEB Meeting on Experimental Biology: Translating the Genome. San Diego, CA, USA. April 11-15, 2003. FASEB.
ISSN: 0892-6638 (ISSN print). Language: English.

AB Native **plasminogen** (Glu-Pg) in the presence of Cl-assumes a tight a-conformation in which the N-terminus is associated with Kringle 5 and the molecule is resistant to Pg activators. Benzamidine (Bz), which binds to Kringle 5, changes the Glu-Pg to the more open ss conformation. Lys-Pg, lacking the first 77 amino acids of Glu-Pg, is thought to be in the ss-conformation naturally. Since the conformation affects the rate of activation of Pg to plasmin, the purpose of this study was to characterize the products produced by neutrophil elastase (NE) in the presence of Cl-and Bz. Glu and Lys Pg were incubated with NE with Bz or Cl-. Incubation mixtures were analyzed by SDS PAGE and Western blots. Glu-Pg and Lys-Pg, digested by NE in the presence of Bz, were completely reduced to low **molecular weight** products by 4 hours. After 4 hours in Cl-buffer most of the full length Glu-Pg remained, whereas the Lys-Pg was digested. Two strong bands (38kDa and 43kDa), miniplasminogen and **angiostatin**, were the main products in all cases. A band of about 66 kDa was seen only in the Bz digests. This band represents a molecule with Kringles 1-5 and at least part of the protease domain. In the presence of Bz Glu-Pg takes on a conformation similar to that of Lys-Pg, which is very susceptible to multiple cleavages by NE. The 66kDa band, seen only when Bz is present, suggests that Bz binding reveals a unique cleavage site for NE in the protease domain.).

L20 ANSWER 16 OF 33 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2002427210 EMBASE **Angiostatin** binds to tyrosine kinase substrate annexin II through the lysine-binding domain in endothelial cells. Tuszynski G.P.; Sharma M.R.; Rothman V.L.; Sharma M.C.. M.C. Sharma, Department of Surgery, MCP Hahnemann University, MS #413, 245 North 15th Street, Philadelphia, PA 19102, United States. ms66@drexel.edu.

Microvascular Research Vol. 64, No. 3, pp. 448-462 2002.
Refs: 58.
ISSN: 0026-2862. CODEN: MIVRA6
Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 20021205
AB **Angiostatin** (AS), an internal fragment of **plasminogen**, is one of the most potent specific inhibitors of angiogenesis. **Angiostatin** treatment has resulted in the complete regression of human tumors implanted subcutaneously into nude mice and has great therapeutic value (O'Reilly et al., Nat. Med. 2, 689-692, 1996). Despite promising therapeutic value in the treatment of cancer, the mechanism of its action is still unknown. We found that **angiostatin** binds to a 35-kDa protein in bovine aortic endothelial (BAE) cells (Sharma et al., Proc. Am. Associate Cancer Res. 42, 568, A3050, 2002). In an attempt to begin to understand **angiostatin**'s mechanism of action, we have purified and characterized this 35-kDa protein from BAE cells. Internal peptide sequence analysis of purified protein demonstrated (SLYYIQQDTK, SYSPYDMLESIK, and ALLYLXGGDD) 100% sequence identity with tyrosine kinase substrate annexin II. Solid phase binding analysis suggests that **angiostatin** specifically bound to purified annexin II immobilized on 96-well plastic plates. Hundred-fold molar excess of unlabeled AS and anti-annexin II antibody inhibited bindings 85 and 55%, respectively, suggesting specific interaction. Annexin II is a predominant receptor for **angiostatin**, since neutralizing the **angiostatin** by soluble receptor (annexin II) effectively blocks **angiostatin**'s anti-EC activity. Similarly, saturating the annexin II receptor by **plasminogen** in endothelial cells also blocks **angiostatin** 's activity. Both **angiostatin** and **plasminogen** bind to purified annexin II in BAE cells saturably with apparent K(d) values of 101 and 164 nM, respectively, for purified annexin II and K(d) values of 83 and 125 nM, respectively, for BAE cells. Anti-annexin II monoclonal antibody inhibited **angiostatin** and **plasminogen** binding to endothelial cells by 68 and 62%, respectively, supporting our in vitro studies that annexin II is a receptor for **angiostatin.** **Angiostatin**-binding protein/annexin II specifically expressed in endothelial cells but not in fibroblasts suggests its EC-specific function. ε-Aminocaproic acid, a lys analogue, effectively blocks **angiostatin** and annexin II interaction, indicating that the lysine-binding domain of AS is required for binding to annexin II. These results suggest that the antiangiogenic action of **angiostatin** may be mediated via interaction with annexin II. Identification of annexin II as a receptor for **angiostatin** provides further evidence that clotting and fibrinolytic pathways are directly involved in the angiogenic process. .COPYRGT. 2002 Elsevier Science (USA).

L20 ANSWER 17 OF 33 MEDLINE on STN DUPLICATE 5
2002311314. PubMed ID: 12032493. **Angiostatin** II is the predominant glycoform in pleural effusates of rabbit VX-2 lung tumors.
Hatton Mark W C; Southward Suzanne M R; Ross Bonnie L; Legault Kimberly; Marien Lindsay; Korbie Darren; Richardson Mary; Singh Gurmit; Clarke Bryan J; Blajchman Morris A. (Department of Pathology and Molecular Medicine, McMaster University Health Sciences Centre, Hamilton, Ontario, Canada.)
Journal of laboratory and clinical medicine, (2002 May) 139 (5) 316-23.
Journal code: 0375375. ISSN: 0022-2143. Pub. country: United States.
Language: English.
AB **Angiostatin** (AST), a polypeptide with potent antiangiogenic properties, is released proteolytically from **plasminogen** in vivo. **Plasminogen** exists naturally in plasma as two glycoforms (PLGs), I and II. Recently it was shown with the use of a chick-embryo chorioallantoic membrane (CAM) assay that rabbit PLG-I and -II yield distinct ASTs-AST-I and -II, respectively-with different antiangiogenic activities. AST glycoforms were of similar **molecular weight**, approximately 30 to 32,000 kD, and probably consisted of kringles 1 to 3 only. AST has now been identified in the interpleural effusate released from VX-2 lung tumors in rabbits. Effusate was

collected from six rabbits with high tumor burdens and fractionated by means of lysine-Sepharose chromatography. The epsilon-aminohexanoic acid-eluted protein of all effusates contained AST (kringles 1-3) at a mean concentration of 1.2 microg/mL of effusate; with regard to AST content, 97% was AST-II. A CAM assay revealed that the lysine-Sepharose-bound fraction from all interpleural effusates contained potent antiangiogenic activity. Blood and urine from rabbits with high burdens of VX-2 contained essentially only AST-II, at mean concentrations of 145 and 4 ng/mL, respectively. AST was absent from the blood of control rabbits. In an attempt to compare their uptake by VX-2, iodine 125-labeled AST-I and iodine 131-labeled AST-II were injected intravenously into tumor-bearing rabbits. AST-I entered the tumor 1.6 times faster than AST-II. As a means of accounting for the preponderance of AST-II in the interpleural effusate, we postulate that VX-2 cells release proteolytic activity to activate **plasminogen** but that of the two PLGs, PLG-II may be the preferred substrate for AST formation in vivo.

L20 ANSWER 18 OF 33 MEDLINE on STN

2002477874. PubMed ID: 12186748. Expression of human endostatin in larvae of silkworm (Bombyx mori) and in vitro activity assays. Yongfeng Jin; Yingfei Wang; Zhenhong Zhu; Yaozhou Zhang. (Institute of Biochemistry, Zhejiang University, Hangzhou, People's Republic of China.) Journal of biochemistry, molecular biology, and biophysics : JBMBB : official journal of the Federation of Asian and Oceanian Biochemists and Molecular Biologists (FAOBMB), (2002 Aug) 6 (4) 293-9. Journal code: 9714994. ISSN: 1025-8140. Pub. country: England: United Kingdom. Language: English.

AB Human endostatin is a novel antiangiogenic molecule, which can inhibit the proliferation and development of new blood vessels, and experimentally can cause nearly complete regression of established tumors. In this paper, the cDNA encoding human endostatin was cloned into a baculovirus shuttle vector pBacPAK8 and co-infected with linearized Bm-BacPAK6 DNA into and BmN cells. The recombinant virus was screened and identified by PCR, DNA and RNA dot hybridization, and ELISA assay. The recombinant endostatin was expressed in culture cells, and the larvae and pupa of silkworm by inoculation of recombinant virus. The biological activity assay showed that the expression product in larvae was over 150 microg/ml, about 50-fold higher than that expressed in cultured cells. SDS-PAGE and Western blotting analysis showed a pattern of **molecular weight** of about 20 kDa. The bio-activity of the protein product was determined by human umbilical vein endothelial cells (ECV304) proliferation test in vitro and the chick chorioallantoic membrane (CAM) vascular inhibition test. Endostatin showed significant inhibitory effect on endothelial cells in a dose-dependent manner. Silkworm-produced endostatin induced apoptosis of endothelial cells and also inhibited angiogenesis in the CAM assay. Combination regimen using **angiostatin** and endostatin showed more than additive effect in angiogenic inhibition and increasing apoptosis when compared with treatment with the individual antiangiogenic protein.

L20 ANSWER 19 OF 33 CAPLUS COPYRIGHT 2005 ACS on STN

2002:758838 Document No. 138:314042 Inhibition of **angiostatin** to the growth and metastasis of gastric cancer in nude mice. Liu, Bing-Ya; Chen, Xue-Hua; Zhu, Zheng-Gang; Lin, Yan-Zhen; Lu, Wei-Xin; Guo, Li-He; Zhu, Li-Hua (Department of Surgery, Shanghai Institute of Digestive Surgery, Ruijin Hospital, SSMU, Shanghai, 200025, Peop. Rep. China). Journal of Shanghai Second Medical University, 14(2), 82-86 (English) 2002. CODEN: JSSUE7. ISSN: 1001-6686. Publisher: Shanghai Second Medical University.

AB Objective: To study the inhibition effect on tumor angiogenesis and metastasis of **angiostatin**, which generated from human **plasminogen.** Methods: **Plasminogen** was isolated from human plasma by Sepharose chromatog. and then catalyzed by elastase. **Angiostatin** was isolated by Sepharose 4B-Lysine chromatog. Nude mice model of metastatic gastric cancer was set up by intact tumor tissue

implantation orthotopically. From the day of operation, mice received daily i.p. injections of human **angiostatin**, intact **plasminogen**, or saline, resp. **Angiostatin** or **plasminogen** [24 μg (1.2 mg/kg)] was given on the day of operation, followed by a daily dose of 12 μg (0.6 mg/kg) via i.p. injection for three weeks. Ten weeks after implantation, mice were sacrificed and autopsied. Microvascular d. was measured by immunohistochem. Results: **Mol. weight** of **plasminogen** isolated from plasma was 94 kDa. **Plasminogen** was catalyzed into two fragment peptides by elastase, which were 41 .apprx. 43 kDa and 51 .apprx. 53 kDa in **mol. weight** Growth of the orthotopically implanted tumor was significantly reduced in size in the mice treated with **angiostatin** with an inhibition rate of 54.0%. Tumor metastasis to the liver and peritoneum was also significantly inhibited by **angiostatin** with inhibition rate of 61.9% and 55.6%, resp. The microvascular d. was also decreased significantly in the **angiostatin** treated mice. Conclusion: **Angiostatin** may be generated from plasma, and has inhibitory effect both on tumor growth and metastasis in nude mice model of human gastric cancer.

L20 ANSWER 20 OF 33 EMBASE on STN

2003458329 EMBASE Purification and characterization of A(61): An **angiostatin**-like **plasminogen** fragment produced by plasmin autodigestion in the absence of sulfhydryl donors. Kassam G.; Kwon M.; Yoon C.-S.; Graham K.S.; Young M.K.; Gluck S.; Waisman D.M.. D.M. Waisman, Dept. of Biochem. and Molec. Biology, Faculty of Medicine, University of Calgary, 3330 Hospital Drive N.W., Calgary, Alta. T2N 4N1, Canada. waisman@ucalgary.ca. Journal of Biological Chemistry Vol. 276, No. 12, pp. 8924-8933 23 Mar 2001.
Refs: 48.
ISSN: 0021-9258. CODEN: JBCHA3
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 20031229

AB Plasmin, a broad spectrum proteinase, is inactivated by an autoproteolytic reaction that results in the destruction of the heavy and light chains of the protein. Recently we demonstrated that a 61-kDa plasmin fragment was one of the major products of this autoproteolytic reaction (Fitzpatrick, S. L., Kassam, G., Choi, K. S., Kang, H. M., Fogg, D. K., and Waisman, D. M. (2000) Biochemistry 39, 1021-1028). In the present communication we have identified the 61-kDa plasmin fragment as a novel four kringle-containing protein consisting of the amino acid sequence Lys(78)-Lys(468). To avoid confusion with the plasmin(ogen) fragment, **angiostatin**.RTM. (Lys(78)-Ala (440)), we have named this protein A(61). Unlike **angiostatin**, A(61) was produced in vitro from plasmin autodigestion in the absence of sulfhydryl donors. A(61) bound to lysine-Sepharose and also underwent a large increase in fluorescence yield upon binding of the lysine analogue, trans-4-aminomethylcyclohexanecarboxy lic acid. Circular dichroism suggested that A(61) was composed of 21% β-strand, β-turn, 18% 3(1)-helix and 8% 3(10)-helix. A(61) was an anti-angiogenic protein as indicated by the inhibition of bovine capillary endothelial cell proliferation. **Plasminogen** was converted to A(61) by HT1080 cells and bovine capillary endothelial cells. Furthermore, a **plasminogen** fragment similar to A(61) was present in the serum of humans as well as normal and tumor-bearing mice. These results establish that plasmin turnover can generate anti-angiogenic plasmin fragments in a non-pathological setting.

L20 ANSWER 21 OF 33 SCISEARCH on STN

2001:855035 The Genuine Article (R) Number: 483FZ. The hemostatic system and angiogenesis in malignancy. Wojtukiewicz M Z; Sierko E; Klement P; Rak J (Reprint). McMaster Univ, Hamilton Civ Hosp, Res Ctr, Dept Med, 711 Concess St, Hamilton, ON L8V 1C3, Canada (Reprint); McMaster Univ,

Hamilton Civ Hosp, Res Ctr, Dept Med, Hamilton, ON L8V 1C3, Canada; Med Acad Bialystok, Dept Oncol, Bialystok, Poland; Univ Vet & Pharmaceut Sci Brno, Fac Vet, Brno, Czech Republic. NEOPLASIA (SEP-OCT 2001) Vol. 3, No. 5, pp. 371-384. ISSN: 1522-8002. Publisher: NATURE AMERICA INC, 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Coagulopathy and angiogenesis are among the most consistent host responses associated with cancer. These two respective processes, hitherto viewed as distinct, may in fact be functionally inseparable as blood coagulation and fibrinolysis, in their own right, influence tumor angiogenesis and thereby contribute to malignant growth. In addition, tumor angiogenesis appears to be controlled through both standard and non-standard functions of such elements of the hemostatic system as tissue factor, thrombin, fibrin, **plasminogen** activators, **plasminogen**, and platelets. "Cryptic" domains can be released from hemostatic proteins through proteolytic cleavage, and act systemically as angiogenesis inhibitors (e.g., **angiostatin**, antiangiogenic antithrombin III aaATIII). Various components of the hemostatic system either promote or inhibit angiogenesis and likely act by changing the net angiogenic balance. However, their complex influences are far from being fully understood. Targeted pharmacological and/or genetic inhibition of pro-angiogenic activities of the hemostatic system and exploitation of endogenous angiogenesis inhibitors of the **angiostatin** and aaATIII variety are under study as prospective anti-cancer treatments.

L20 ANSWER 22 OF 33 CAPLUS COPYRIGHT 2005 ACS on STN
2001:351110 Document No. 135:222039 Cloning, expression, purification and identification of kringle 5 domain of human **plasminogen.** Chen, Hao; Chen, Yuhong; Zhang, Jing; Liu, Jianning; Zhu, Dexu (Inst. Molecular Med., Nanjing Univ., Nanjing, 210093, Peop. Rep. China). Nanjing Daxue Xuebao, Ziran Kexue, 37(2), 218-222 (Chinese) 2001. CODEN: NCHPAZ. ISSN: 0469-5097. Publisher: Nanjing Daxue.

AB **Angiostatin** is a potent angiogenesis inhibitor which has been identified as an internal fragment of **plasminogen** that includes its first four kringle modules. The kringle 5 domain of human **plasminogen** would appear to be more potent than **angiostatin** on inhibition of basic fibroblast growth factor-stimulated capillary endothelial cell proliferation. The gene-encoding for kringle 5 domain of human **plasminogen** was obtained by PCR using human **plasminogen** cDNA as template. The amplified fragment was cloned into the vector pET25b(+) to construct the recombinant expression vector. Upon induction with IPTG, the Escherichia coli BL21(DE3) containing the recombinant plasmid could express a distinct band with a **mol. weight** of 12 kD. Most of the kringle 5 was expressed in the form of the inclusion body without biol. activity. The inclusion body was refolded in vitro and purified with SP-Sepharose FF ion-exchange chromatog. After single step elution, the sample was purified and it showed one band by 15% SDS-PAGE anal., which was, detected by Coomassie brilliant blue stain. The purity of protein is more than 95%. The target protein also showed high activity of inhibition to bovine capillary endothelial cell proliferation which was induced by bFGF.

L20 ANSWER 23 OF 33 MEDLINE on STN DUPLICATE 6
2001641604. PubMed ID: 11694280. Aminoalkyl affinity matrices. Houen G. (Department of Research and Development, Division of Biologicals, Statens Serum Institut, Artillerivej 5, DK-2300, Copenhagen, Denmark.. gh@ssi.dk) . Journal of biochemical and biophysical methods, (2001 Oct 30) 49 (1-3) 189-97. Journal code: 7907378. ISSN: 0165-022X. Pub. country: Netherlands. Language: English.

AB Aminoalkyl matrices are used in affinity chromatography of amine oxidases and other proteins with affinity for amino groups. Under appropriate circumstances chromatography on aminoalkyl matrices may yield purification factors around 100 to 1000, and they have been used in affinity purification of many members of the amine oxidase family. Other proteins with affinity for aminoalkyl matrices include thiol ester proteins,

lactoferrin, and proteins with lysine-binding kringles (**plasminogen**, **plasminogen** activator, apolipoprotein A). The affinity of thiol ester proteins for aminoalkyl matrices is abolished after inactivation of the thiol ester group by reaction with low **molecular weight** amines including ammonia. Due to this, an ammonium sulphate precipitation step should be included in purification schemes for amine oxidases. The affinity of lactoferrin for aminoalkyl matrices stems from an affinity for the repeating amino groups in glycosaminoglycans, and this explains why lactoferrin requires diamines for efficient elution. The affinity of **plasminogen** for aminoalkyl groups is exploited in a one-step purification from plasma, and is also utilised in purification schemes for **angiostatin**, an angiogenesis-inhibiting fragment of **plasminogen.** Apolipoprotein A is homologous to **plasminogen**, and also has affinity for aminohexyl columns. The common binding motif for these proteins are lysine-binding kringles. Due to the properties of the amino group itself, aminoalkyl matrices will inevitably also function as anion exchangers, and this must be taken into consideration in the choice of conditions for sample loading, column washing and elution of bound proteins. Depending on the length of the alkyl chain, the matrices also have a potential for hydrophobic interactions. This property has been exploited in the purification of several proteins but must be minimized during affinity chromatography of amine oxidases. In conclusion, aminoalkyl matrices are valuable tools for affinity chromatography of several different proteins, and simple variations of sample pretreatment, sample loading, and column washing and elution conditions allow efficient selective purification of proteins with different affinities for the matrices.

L20 ANSWER 24 OF 33 CAPLUS COPYRIGHT 2005 ACS on STN

2000:741951 Document No. 133:313601 Kringle domains of **plasminogen** capable of modulating angiogenesis in vivo. Cao, Yihai (Karolinska Innovations AB, Swed.). PCT Int. Appl. WO 2000061179 A1 20001019, 79 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-SE719 20000414. PRIORITY: US 1999-291200 19990414.

AB The present invention relates to compns. and proteins capable of regulating endothelial cell proliferation as well as various uses thereof. The compns. and proteins are characterized by inclusion of amino acid sequences associated with mammalian Kringles 1, 2 and/or 3 together with that of Kringle 5 or consist of anti-angiogenically active peptide fragments or protected derivs. thereof. The protein according to the invention has a **mol. weight** of approx. 50-75, such as 50-60 kDa, preferably about 55 kDa, and has an amino acid sequence, which is substantially similar to that of a **plasminogen** fragment comprised of Lys 78-Arg 530 of a **plasminogen** mol.

L20 ANSWER 25 OF 33 CAPLUS COPYRIGHT 2005 ACS on STN

2000:384237 Document No. 133:38210 Compositions and methods of using proteins and peptides that bind angiogenesis-inhibiting proteins. MacDonald, Nicholas J.; Sim, Kim Lee (Entremed, Inc., USA). PCT Int. Appl. WO 2000032631 A2 20000608, 100 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US28897 19991206. PRIORITY: US 1998-206059 19981204.

AB The present invention relates to peptides and proteins such as receptors that bind angiogenesis-related proteins **Angiostatin** protein and/or Endostatin protein. Peptides and proteins of the present invention can be isolated from body fluids including blood or urine, or can be synthesized by recombinant, enzymic or chemical methods. The peptides are particularly important for identifying receptors of angiogenesis-related proteins, as well as for identifying other proteins that regulate, transport and otherwise interact with angiogenesis-related proteins. The present invention in particular relates to laminin protein as a putative receptor for **Angiostatin** protein and tropomyosin as a putative Endostatin protein.

L20 ANSWER 26 OF 33 CAPLUS COPYRIGHT 2005 ACS on STN
2000:241459 Document No. 132:275964 Novel human aspartase homologous to cathepsin D precursor and use for producing anti-metastasis plasma protein fragments. Morikawa, Wataru; Kaminaka, Kazuyoshi; Takemoto, Sumiyo; Maeda, Hiroaki; Nozaki, Chikateru; Miyamoto, Seiji (Juridical Foundation the Chemo-Sero-Therapeutic Research Institute, Japan). PCT Int. Appl. WO 2000020570 A1 20000413, 55 pp. DESIGNATED STATES: W: US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (Japanese). CODEN: PIXXD2. APPLICATION: WO 1999-JP5322 19990929. PRIORITY: JP 1998-296095 19981002.

AB A novel aspartase, PACE4 (**plasminogen angiostatin** converting enzyme of pH 4), is prepared from cell line PC-3 that was established from human prostate cancer and characterized. PACE4 exhibits a **mol. weight** of 45 kDa as determined by non-reducing SDS-PAGE and LVRIPLHKFT at the N-terminus. PACE4 aspartase is highly homol. to human cathepsin D precursor and can degrade plasma proteins such as **plasminogen**, fibronectin, vitronectin, and human hepatic growth factor into fragments that have the **angiostatin**-like activities and thus the anti-metastasis effects. A pharmaceutical composition containing PACE4 for the prevention of treatment of solid cancers, diabetic retinopathy, or rheumatism is also claimed.

L20 ANSWER 27 OF 33 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2000:510238 The Genuine Article (R) Number: 329QF. Tetranectin-binding site on **plasminogen** kringle 4 involves the lysine-binding pocket and at least one additional amino acid residue. Graversen J H; Sigurskjold B W; Thogersen H C; Etzerodt M (Reprint). Aarhus Univ, Dept Biol Mol & Struct, Gene Express Lab, DK-8000 Aarhus C, Denmark (Reprint); Univ Copenhagen, August Krogh Inst, DK-2100 Copenhagen, Denmark. BIOCHEMISTRY (27 JUN 2000) Vol. 39, No. 25, pp. 7414-7419. ISSN: 0006-2960. Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Kringle domains are found in a number of proteins where they govern protein-protein interactions. These interactions are often sensitive to lysine and lysine analogues, and the kringle-lysine interaction has been used as a model system for investigating kringle-protein interactions. In this study, we analyze the interaction of wild-type and six single-residue mutants of recombinant **plasminogen** kringle 4 expressed in Escherichia coli with the recombinant C-type lectin domain of tetranectin and trans-aminomethyl-cyclohexanoic acid (t-AMCHA) using isothermal titration calorimetry. We find that all amino acid residues of **plasminogen** kringle 4 found to be involved in r-AMCHA binding are also involved in binding tetranectin. Notably, one amino acid residue of **plasminogen** kringle 4, Arg 32, not: involved in binding t-AMCHA, is critical for binding tetranectin. We also find that Asp 57 and Asp 55 of **plasminogen** kringle 4, which both were found to interact with the low **molecular weight** ligand with an almost: identical geometry in the crystal of the complex, are not of equal functional importance in t-AMCHA binding. Mutating Asp 57 to an Asn totally eliminates binding, whereas the Asp 55 to Asn, like the Arg 71 to Gin mutation, was found only to decrease affinity.

L20 ANSWER 28 OF 33 MEDLINE on STN DUPLICATE 7
2001070587. PubMed ID: 11095991. Human glioma cell BT325 expresses a proteinase that converts human **plasminogen** to kringle 1-5-containing fragments. Li F; Yang J; Liu X; He P; Ji S; Wang J; Han J; Chen N; Yao L. (Department of Biochemistry and Molecular Biology, Fourth Military Medical University, Xi'an, 710032, People's Republic of China.) Biochemical and biophysical research communications, (2000 Nov 30) 278 (3) 821-5. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB **Angiostatin**, a specific angiogenesis inhibitor, is an internal fragment of **plasminogen**, and can be generated in many systems mediated by different enzymes in vitro. The mechanism of **angiostatin** generation in vivo has not been well defined. Here we demonstrated that human glioma cell line BT325 can express an enzyme that can convert purified **plasminogen** to **angiostatin**-like fragments with molecular masses of 65, 60, and 58 kDa, respectively. These fragments have an identical N-terminal as KVYLS, which starts from Lys(98) of the **plasminogen** precusor. According to their molecular mass, the three fragments should comprise kringle domain 1 to kringle domain 5 (kringle 1-5). The proteolytic fragments obtained as above can inhibit the growth of bovine aortic endothelial (BAE) cells specifically. The proteolysis process can be completely inhibited by serine proteinase inhibitors, and partially inhibited by EDTA. The **molecular weight** of the peptide, which contains an enzymatic activity responsible for the proteolysis, was 13 kD determined by gel filtration and SDS-PAGE. The present data suggest that glioma cell BT325 can produce a novel proteinase to generate kringle 1-5 of **plasminogen** as an angiogenesis inhibitor.

L20 ANSWER 29 OF 33 MEDLINE on STN
1999233566. PubMed ID: 10215610. Extrahepatic synthesis of **plasminogen** in the human cornea is up-regulated by interleukins-1alpha and -1beta. Twining S S; Wilson P M; Ngamkitidechakul C. (Departments of Biochemistry and Ophthalmology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA.. stwining@mcw.edu) . Biochemical journal, (1999 May 1) 339 (Pt 3) 705-12. Journal code: 2984726R. ISSN: 0264-6021. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The avascular cornea has limited access to plasma proteins, including **plasminogen**, a protein that is synthesized by the liver and supplied to most tissues via the blood. Recent experiments by others using **plasminogen**-deficient mice revealed the importance of plasmin, the active form of **plasminogen**, for the maintenance of the normal cornea and for corneal wound healing [Kao, Kao, Bugge, Kaufman, Kombrinck, Converse, Good and Degan (1998) Invest. Ophthalmol. Vis. Sci. 39, 502-508; Drew, Kaufman, Kombrinck, Danton, Daugherty, Degen and Bugge (1998) Blood 91, 1616-1624]. In the present experiments, plasmin was identified as a major serine proteinase in the human cornea. The major **plasminogen** and plasmin forms on non-reducing zymograms and Western blots had Mr values of 76x10(3) and 85x10(3), with minor forms of Mr 200x10(3), 135x10(3), 68x10(3) and 45x10(3). **Angiostatin** -like peptides with Mrs of 48x10(3), 45x10(3) and 38x10(3) were observed which bound to lysine-Sepharose and reacted with anti-**plasminogen** monoclonal antibodies directed towards kringle domains 1-3 of **plasminogen.** The cornea contained 1.1+/-0.15 microgram of **plasminogen**+plasmin/cornea, or 0.54+/-0.05 microgram of **plasminogen**+plasmin/mg of protein. Cornea conditioned medium contained nine times the amount of **plasminogen**+plasmin that could be extracted from the cornea. These data suggested that corneal cells, unlike most extrahepatic cells, synthesize **plasminogen.** The synthesis of **plasminogen** by the cornea was confirmed by immunoprecipitation of metabolically labelled **plasminogen**, sequencing of its cDNA obtained by reverse transcriptase-PCR and

inhibition of protein synthesis. Interleukins-1alpha and -1beta stimulated corneal **plasminogen** synthesis 2-3-fold; however, interleukin-6 decreased corneal **plasminogen** synthesis by approx. 40% at early times after addition of the cytokine. By 24 h of culture, no differences were noted in the presence and absence of interleukin-6. Thus the cornea can synthesize **plasminogen** and regulate its synthesis in response to its environment, including cytokines induced in the cornea by injury and inflammation. Therefore the cornea can control the amount of **plasminogen**, the precursor of both plasmin and **angiostatin.**

L20 ANSWER 30 OF 33 CAPLUS COPYRIGHT 2005 ACS on STN
1998:545400 Document No. 129:170983 sequence of mouse **angiostatin** protein with detection methods and applications to inhibit endothelial cell proliferation and cancer. O'Reilly, Michael S.; Folkman, M. Judah (The Children's Medical Center Corp., USA). U.S. US 5792845 A 19980811, 39 pp., Cont.-in-part of U. S. 5,639,725. (English). CODEN: USXXAM. APPLICATION: US 1994-326785 19941020. PRIORITY: US 1994-248629 19940426.
AB The endothelial inhibitor is a protein isolated from the blood or urine that is eluted as a single peak from C4-reverse phase high performance liquid chromatog. The endothelial inhibitor is a mol. comprising a protein having a **mol. weight** of between approx. 38 kilodaltons and 45 kilodaltons as determined by reducing polyacrylamide gel electrophoresis and having an amino acid sequence substantially similar to that of a murine **plasminogen** fragment beginning at amino acid number 98 of a murine **plasminogen** mol. Diagnostic assays and kits for **angiostatin** measurement, and histochem. kits for localization of **angiostatin**, and mol. probes to monitor **angiostatin** biosynthesis, and antibodies specific for **angiostatin** are all described.

L20 ANSWER 31 OF 33 MEDLINE on STN DUPLICATE 8
97471284. PubMed ID: 9330225. Limited proteolysis of angiogenin by elastase is regulated by **plasminogen.** Hu G F. (Center for Biochemical and Biophysical Sciences and Medicine, Harvard Medical School, Boston, Massachusetts 02115, USA.. guofuhu@warren.med.harvard.edu) . Journal of protein chemistry, (1997 Oct) 16 (7) 669-79. Journal code: 8217321. ISSN: 0277-8033. Pub. country: United States. Language: English.
AB Human neutrophil elastase cleaves angiogenin at the Ile-29/Met-30 peptide bond to produce two major disulfide-linked fragments with apparent **molecular weights** of 10,000 and 4000, respectively. Elastase-cleaved angiogenin has slightly increased ribonucleolytic activity, but has lost its ability to undergo nuclear translocation in endothelial cells, a process essential for angiogenic activity. Cleavage appears to alter the cell-binding properties of angiogenin, despite the fact that it occurs some distance from the putative receptor-binding site, since the elastase-cleaved protein fails to compete with its native counterpart for nuclear translocation in endothelial cells. **Plasminogen** specifically accelerates elastase proteolysis of angiogenin. It does not enhance elastase activity toward ribonuclease A or the synthetic peptide substrate MeOSuc-Ala-Ala-Pro-Val-pNA. **Plasminogen**-accelerated inactivation of angiogenin by elastase might be a significant event in the process of angiogenin-induced angiogenesis since (i) angiogenin and **plasminogen** circulate in plasma at high concentrations, (ii) angiogenin, especially when bound to actin, activates tissue **plasminogen** activator to generate plasmin from **plasminogen**, and (iii) elastase cleaves **plasminogen** to produce **angiostatin**, a potent inhibitor of angiogenesis and metastasis. Interrelationships among angiogenin, **plasminogen**, **plasminogen** activators, elastase, and **angiostatin** may provide a sensitive regulatory system to balance angiogenesis and antiangiogenesis.

L20 ANSWER 32 OF 33 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 9

97000517 EMBASE Document No.: 1997000517. Angiogenesis inhibition as a drug target for disease: An update. Seed M.P.. M.P. Seed, Dept. of Experimental Pathology, William Harvery Research Institute, Royal School of Medicine/Dentistry, Charterhouse Square, London EC1M 6BQ, United Kingdom. Expert Opinion on Investigational Drugs Vol. 5, No. 12, pp. 1617-1637 1996.
ISSN: 1354-3784. CODEN: EOIDER
Pub. Country: United Kingdom. Language: English. Summary Language: English.
ED Entered STN: 970215
AB Angiogenesis is required for the development of many proliferative diseases, including granulomatous disease, such as rheumatoid arthritis, psoriasis and neoplasia, as well as diabetic retinopathy. A substantial effort is being made to develop inhibitors of angiogenesis for the treatment of these diseases. This article is an update of a previous review [Colville-Nash and Seed, Curr. Opin. Invest. Drugs (1993) 2:763-813], and reviews the recent developments in the use of: angiostatic steroids, fumagillol derivatives, somatostatin analogues, matrix metalloproteinase (MMP) inhibitors, modulators of vascular endothelial cell growth factor (VEGF), fibroblast growth factor (FGF), **angiostatin**, endostatin, platelet factor-4 (PF4), thrombospondin-1 (TSP-1), cell adhesion molecules (integrins and selectins), urokinase **plasminogen** receptor antagonists, cyclo-oxygenase (COX) and non-steroidal anti-inflammatory drugs (NSAIDs), nitric oxide synthase (NOS), cytokine-suppressing anti-inflammatory drugs (CSAIDs), and drug combinations. Most of these approaches have been shown to be effective in inhibiting tumour growth in vivo, and many in models of inflammation. The field has, therefore, a very wide range of effective drug targets which are being exploited. Many areas are still limited by their reliance on high **molecular weight** molecular technologies, antibodies and constructs; however, low **molecular weight** compounds are now being sought in areas such as cytokine suppression, VEGF, MMPs, COX, NOS, and adhesion molecules. Angiostatic therapy is a rapidly advancing, therapeutically viable and exciting field.

L20 ANSWER 33 OF 33 CAPLUS COPYRIGHT 2005 ACS on STN
1995:998155 Document No. 124:76505 **Angiostatin** and method of use for inhibition of angiogenesis and cancer treatment. Ooreilly, Michael S.; Folkman, M. Judah; Sim, Kim Lee; Cao, Yihai (Children's Medical Center Corp., USA). PCT Int. Appl. WO 9529242 A1 19951102, 123 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2.
APPLICATION: WO 1995-US5107 19950426. PRIORITY: US 1994-248629 19940426; US 1994-326785 19941020.
AB The present invention comprises an endothelial inhibitor and method of use therefor. The endothelial inhibitor is a protein isolated from the blood or uring that is eluted as a single peak from C4-reverse phase high performance liquid chromatog. The endothelial inhibitor is a mol. comprising a protein having a **mol. weight** o between approx. 38 kilodaltons and 45 kiladaltons as determined by a reducing polyacylamide gel electrophoresis and having an amino acid sequence substantially similar to that of a murine **plasminogen** fragment beginning at amino acid number 98 of a murine **plasminogen** mol.

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L21 29 (MULLIGAN-KEHOE M?/AU)

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L22 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
2004:513314 Document No. 141:65104 Methods using a recombinant plasminogen activator inhibitor-1 (rPAI-1) isoform for modulating angiogenesis. **Mulligan-Kehoe, Mary Jo** (USA). U.S. Pat. Appl. Publ. US 2004121955 A1 20040624, 22 pp., Cont.-in-part of WO 2003 84,483. (English). CODEN: USXXCO. APPLICATION: US 2003-686428 20031014. PRIORITY: US 2002-PV369392 20020401; US 2003-PV448301 20030214; WO 2003-US9981 20030401.

AB Recombinant plasminogen activator inhibitor-1 (rPAI-1) isoforms which lack the reactive center loop and contain the complete heparin-binding domain or lack at least a portion of the heparin-binding domain are described. The discloses rPAI-1 isoforms may be used to modulate angiogenesis through blocking release of VEGF from a VEGF-heparin complex. Furthermore, the rPAI-1 proteins may be used to inhibit cell proliferation and migration, induce apoptosis, and produce proteolytic fragments corresponding to angiostatin kringles 1-3 and kringles 1-4. A truncated proteolytic plasmin protein of 34 kDa is also provided.

L22 ANSWER 2 OF 15 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2005:609151 The Genuine Article (R) Number: 866OT. Disruption of synectin gene expression stimulates endothelial cell adhesion while inhibiting FGF2-dependent cell growth, migration, and tube branching. Chittenden T W (Reprint); **Mulligan-Kehoe M J**; Lanahan A; Rhodes J M; Simons M. Dartmouth Coll Sch Med, Lebanon, NH USA. CIRCULATION (26 OCT 2004) Vol. 110, No. 17, Supp. [S], pp. 218-218. MA 1044. ISSN: 0009-7322. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English.

L22 ANSWER 3 OF 15 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2005:608929 The Genuine Article (R) Number: 866OT. Synectin gene disruption produces global vascular defects that associate with impaired cardiac function in adult mice. Chittenden T W (Reprint); Lanahan A; Drinane M; Zhang Z W; Moodie K; deMuinck E D; Helisch A; Palac R T; **Mulligan-Kehoe M J**; Dedkov E; Tomanek R; Simons M. Dartmouth Coll Sch Med, Lebanon, NH USA; Univ Iowa, Iowa City, IA 52242 USA. CIRCULATION (26 OCT 2004) Vol. 110, No. 17, Supp. [S], pp. 172-172. MA 822. ISSN: 0009-7322. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English.

L22 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
2003:818238 Document No. 139:302079 Recombinant plasminogen activator inhibitor-1 (PAI-1) isoforms in methods for modulating angiogenesis via VEGF. **Mulligan-Kehoe, Mary Jo**; Powell, Richard J. (Trustees of Dartmouth College, USA). PCT Int. Appl. WO 2003084483 A2 20031016, 71 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US9981 20030401. PRIORITY: US 2002-PV369392 20020401; US 2003-PV448301 20030214.

AB Recombinant plasminogen activator inhibitor-1 (PAI-1) isoforms which lack the reactive center loop and contain the complete heparin-binding domain or lack at least a portion of the heparin-binding domain are described. The rPAI-1 isoforms disclosed herein may be used to modulate angiogenesis through blocking release of VEGF from a VEGF-heparin complex. Furthermore the rPAI-1 proteins may be used to inhibit cell proliferation and migration, induce apoptosis, and produce proteolytic fragments corresponding to angiostatin kringles 1-3 and kringles 1-4.

L22 ANSWER 5 OF 15 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2003:560544 Document No.: PREV200300561395. The functions of cleaved PAI-1 in angiogenic mechanisms. **Mulligan-Kehoe, Mary Jo** [Reprint Author]. Department of Surgery, Vascular Section, Dartmouth Medical School, Dartmouth College, 1 Medical Center Drive, Borwell 530 E, Lebanon, NH, 03748, USA. International Journal of Molecular Medicine, (2003) Vol. 12, No. Supplement 1, pp. S77. print.
Meeting Info.: 8th World Congress on Advances in Oncology and 6th International Symposium on Molecular Medicine. Crete, Greece. October 16-18, 2003.
ISSN: 1107-3756 (ISSN print). Language: English.

L22 ANSWER 6 OF 15 MEDLINE on STN DUPLICATE 1
2002709220. PubMed ID: 12381729. A truncated plasminogen activator inhibitor-1 protein blocks the availability of heparin-binding vascular endothelial growth factor A isoforms. **Mulligan-Kehoe Mary Jo**; Kleinman Hynda K; Drinane Mary; Wagner Robert J; Wieland Courtney; Powell Richard J. (Department of Surgery, Vascular Surgery Section, Dartmouth Medical School, Dartmouth College, Hanover, New Hampshire 03756, USA.. mary.j.mulligan-kehoe@dartmouth.edu) . Journal of biological chemistry, (2002 Dec 13) 277 (50) 49077-89. Electronic Publication: 2002-10-14. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB We have made deletions of the porcine plasminogen activator inhibitor-1 (PAI-1) gene to obtain recombinant truncated PAI-1 proteins to examine functions of the PAI-1 isoforms. We previously reported that one recombinant truncated protein, rPAI-1(23), induces the formation of angiostatin by cleaving plasmin. The rPAI-1(23) protein is also able to bind urokinase plasminogen activator and plasminogen and then reduce the amount of plasmin that is formed. We have now prepared three different truncated rPAI-1 proteins and demonstrate that PAI-1 conformations control the release of heparin-binding vascular endothelial growth factor (VEGF) isoforms. The rPAI-1(23) isoform can regulate the functional activity of heparan sulfate-binding VEGF-A isoforms by blocking the activation of VEGF from heparan sulfate. The rPAI-1(23) conformation induced extensive apoptosis in cultured endothelial cells and thus reduced the number of proliferating cells. The rPAI-1(23) isoform inhibited migration of VEGF-stimulated sprouting from chick aortic rings by 65%, thus displaying a role in anti-angiogenic mechanisms. This insight into anti-angiogenic functions related to PAI-1 conformational changes could provide potential intervention points in angiogenesis associated with atherosclerotic plaques and cancer.

L22 ANSWER 7 OF 15 MEDLINE on STN DUPLICATE 2
2002352870. PubMed ID: 12096275. The effect of endothelial cell overexpression of plasminogen activator inhibitor-1 on smooth muscle cell migration. Proia Richard R; Nelson Peter R; **Mulligan-Kehoe Mary Jo** ; Wagner Robert J; Kehas Arthur J; Powell Richard J. (Department of Surgery, Dartmouth-Hitchcock Medical Center, Dartmouth Medical School, Lebanon, NH 03756, USA.) Journal of vascular surgery : official publication, Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter, (2002 Jul) 36 (1) 164-71. Journal code: 8407742. ISSN: 0741-5214. Pub. country: United States. Language: English.

AB INTRODUCTION: Plasminogen activator inhibitor-1 (PAI-1), a known inhibitor of plasminogen activators, may regulate smooth muscle cell migration (SMC) through alteration in matrix metalloproteinase (MMP) activity. METHODS: To study the effect of endothelial cell (EC) PAI-1 overexpression on SMC migration, RT-PCR was used to clone the full length PAI-1 gene, which was ligated into the pCMV/myc/ER expression vector. With electroporation, bovine aortic ECs were transfected with either the PAI-1 construct or the empty vector as control. EC PAI-1 overexpression was shown with a specific PAI-1 activity assay and enzyme-linked immunosorbent assay. The effect of EC PAI-1 overexpression on SMC migration was measured with a

modified Boyden-chamber assay. SMC MMP expression was measured with zymography. RESULTS: Selected clones (EC9, EC21) had a three-fold to five-fold increase in PAI-1 activity compared with untransfected EC and empty vector EC (ECC). Similarly, enzyme-linked immunosorbent assay results showed a 3.5-fold to 5.5-fold increase in PAI-1 levels in EC9 and EC21 versus ECC. Untransfected EC and ECC had similar effects on SMC migratory patterns. Migration of SMC exposed to PAI-1 overexpressing EC was inhibited by 35% to 57% compared with ECC. This inhibitory effect was reversed with addition of exogenous urokinase-type plasminogen activator (uPA). Zymography showed downregulation of MMP-2 and MMP-9 in SMCs exposed to PAI-1 overexpressing EC. CONCLUSION: PAI-1 overexpression with transfected EC inhibits SMC migration. This effect may be mediated through decreased SMC MMP activity.

L22 ANSWER 8 OF 15 MEDLINE on STN DUPLICATE 3

2001290742. PubMed ID: 11113116. A truncated plasminogen activator inhibitor-1 protein induces and inhibits angiostatin (kringles 1-3), a plasminogen cleavage product. **Mulligan-Kehoe M J**; Wagner R; Wieland C; Powell R. (Division of Vascular Surgery, Department of Surgery, Dartmouth Medical School, Dartmouth College, Hanover, New Hampshire 03756, USA.. mary.j.mulligan-kehoe@dartmouth.edu) . Journal of biological chemistry, (2001 Mar 16) 276 (11) 8588-96. Electronic Publication: 2000-12-11. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Plasminogen activator inhibitor-1 (PAI-1) is a serpin protease inhibitor that binds plasminogen activators (uPA and tPA) at a reactive center loop located at the carboxyl-terminal amino acid residues 320-351. The loop is stretched across the top of the active PAI-1 protein maintaining the molecule in a rigid conformation. In the latent PAI-1 conformation, the reactive center loop is inserted into one of the beta sheets, thus making the reactive center loop unavailable for interaction with the plasminogen activators. We truncated porcine PAI-1 at the amino and carboxyl termini to eliminate the reactive center loop, part of a heparin binding site, and a vitronectin binding site. The region we maintained corresponds to amino acids 80-265 of mature human PAI-1 containing binding sites for vitronectin, heparin (partial), uPA, tPA, fibrin, thrombin, and the helix F region. The interaction of "inactive" PAI-1, rPAI-1(23), with plasminogen and uPA induces the formation of a proteolytic protein with angiostatin properties. Increasing amounts of rPAI-1(23) inhibit the proteolytic angiostatin fragment. Endothelial cells exposed to exogenous rPAI-1(23) exhibit reduced proliferation, reduced tube formation, and 47% apoptotic cells within 48 h. Transfected endothelial cells secreting rPAI-1(23) have a 30% reduction in proliferation, vastly reduced tube formation, and a 50% reduction in cell migration in the presence of VEGF. These two studies show that rPAI-1(23) interactions with uPA and plasminogen can inhibit plasmin by two mechanisms. In one mechanism, rPAI-1(23) cleaves plasmin to form a proteolytic angiostatin-like protein. In a second mechanism, rPAI-1(23) can bind uPA and/or plasminogen to reduce the number of uPA and plasminogen interactions, hence reducing the amount of plasmin that is produced.

L22 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

2001:119856 Document No. 135:105763 Endothelial cell overexpression of plasminogen activator inhibitor-1 inhibits smooth muscle cell migration. Proia, Richard R.; Nelson, Peter R.; **Mulligan-Kehoe, Mary Jo**; Wagner, Robert J.; Kehas, Arthur J.; Powell, Richard J. (Section of Vascular Surgery, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA). Surgical Forum, 51, 371-373 (English) 2000. CODEN: SUFOAX. ISSN: 0071-8041. Publisher: American College of Surgeons.

AB The intimal hyperplastic response seen in human arteries following vessel wall injury has been well described and is mediated predominantly by vascular smooth muscle cells (SMCs). In the development of hyperplasia lesions, SMCs must migrate to the subintimal space, where they proliferate and produce extracellular matrix. Others have shown that plasminogen activator inhibitor-1 (PAI-1) may favorably affect vascular wall

remodeling following injury. To further define the role of PAI-1 in this process, a PAI-1-overexpressing endothelial cell (EC) line was developed to examine the effect of PAI-1 on SMC migration and SMC matrix metalloproteinase (MMP) activity. Results show that EC PAI-1 overexpression inhibits SMC migration. The effect of PAI-1 overexpression on SMC migration may be due to decreased SMC MMP activity. The mechanism of PAI-1 regulation of SMC MMP activity may be mediated through PAI-1 downregulation of plasmin, a known activator of MMPs. Overexpression of PAI-1 may have a role in favorably affecting the intimal hyperplastic response following arterial injury.

L22 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
1999:795956 Document No. 132:31770 Composition and methods for modulating proteolytic degradation of intracellular targets using a phage display proteasome target library. **Mulligan-Kehoe, Mary Jo** (Government of the United States of America, Represented by the Secretary, Department of Health and Human Services, USA). PCT Int. Appl. WO 9964583 A2 19991216, 53 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US13301 19990611. PRIORITY: US 1998-96899 19980612.
AB The invention relates to the use a target-specific binding protein to inactivate intracellular target by a mechanism involving proteasomal degradation A phage-display library that expresses single-chain recombinant binding proteins is disclosed. Inserts in the library comprise Ig heavy chain framework regions flanking highly divergent, synthetically produced hypervariable regions. In the exemplary case presented, the activity of intracellular target, glucose-6-phosphate dehydrogenase (G6PD), was inhibited by intracellular expression of a cloned single-chain recombinant binding protein. Sequences and constructs for delivering binding proteins to proteasomes and modulating the rate of proteolysis also are disclosed. Methods of inactivating intracellular targets using the recombinant binding proteins are also disclosed.

L22 ANSWER 11 OF 15 MEDLINE on STN DUPLICATE 4
1999272553. PubMed ID: 10339404. Inhibition of cytoplasmic antigen, glucose- 6-phosphate dehydrogenase, by VH-CH1, an intracellular Fd fragment antibody derived from a semisynthetic Fd fragment phage display library. **Mulligan-Kehoe M J**; Russo A. (National Cancer Institute, National Institutes of Health, Bethesda, MD, USA.. mary.j.mulligan-kehoe@dartmouth.edu) . Journal of molecular biology, (1999 May 28) 289 (1) 41-55. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.
AB A library of Fd fragment antibody binding proteins was created by random mutation of 15 nucleotides within the CDRIII region of the immunoglobulin heavy chain gene and displayed as Fd coat protein fusion constructs of M13 phage. The library was screened for those VHbinding sites that bound glucose-6-phosphate dehydrogenase (G6PD). One isolate (DH27bp) inhibited G6PD activity by 85 %. The DH27bpgene was re-engineered, placed in a eukaryotic expression vector having an isopropyl-beta-delta-thiogalactopyranoside (IPTG) inducible promoter, and transfected and then expressed in Chinese hamster V79 cells. G6PD activity was completely inhibited. Removal of IPTG reverted the cell to full G6PD activity. The intracellular dynamics of the G6PD/DH27bpcomplex showed that when the proteasomes of cells expressing DH27bpwere inhibited (N -acetyl-Leu-Leu-norleucinal or lactacystin) G6PD activity increased. Metabolic labelling of newly synthesized IPTG-induced proteins during/absence of proteasomal inhibitors showed that both G6PD and DH27bpare signaled for degradation when the intracellular complex is formed. Furthermore, semi-quantitative RT/PCR demonstrated that G6PD mRNA

is upregulated over the time course of G6PD inactivation by DH27bpFd binding protein. These effects were not observed in those cells expressing a non-mutated Fd (UMHC) or in IPTG-treated non-transduced V79 cells. Our results demonstrate that an Fd-based intracellular binding protein can find and disable the function of a specific intracellular target and once the Fd expression is repressed the activity of intracellular targeted protein can revert to normal.

L22 ANSWER 12 OF 15 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2002:126661 Document No.: PREV200200126661. Phage-display of immunoglobulin heavy chain libraries for identification of inhibitors of intracellular constituents. **Mulligan-Kehoe, M. J.** [Inventor]. Springfield, Va., USA. ASSIGNEE: THE UNITED STATES OF AMERICA AS REPRESENTED BY THE DEPARTMENT OF HEALTH AND HUMAN SERVICES. Patent Info.: US 5824520 19981020. Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 20, 1998) Vol. 1215, No. 3, pp. 2981. print.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

L22 ANSWER 13 OF 15 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2002:88528 Document No.: PREV200200088528. Phage-display of immunoglobulin heavy chain libraries. **Mulligan-Kehoe, M. J.** [Inventor]. Springfield, Va., USA. ASSIGNEE: THE UNITED STATES OF AMERICA AS REPRESENTED BY THE DEPARTMENT OF HEALTH AND HUMAN SERVICES. Patent Info.: US 5702892 19971230. Official Gazette of the United States Patent and Trademark Office Patents, (Dec. 30, 1997) Vol. 1205, No. 5, pp. 3854-3855. print.
Language: English.

L22 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

1997:9317 Document No. 126:27677 Phage display library comprising M13-derived vector expressing Ig-like single-chain fusion protein and use for intracellular component inhibition. **Mulligan-Kehoe, Mary Jo** (United States Dept. of Health and Human Services, USA). PCT Int. Appl. WO 9635781 A1 19961114, 27 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US6537 19960508. PRIORITY: US 1995-437815 19950509.

AB One aspect of the invention relates to a phage-display library that expresses single-chain recombinant binding proteins. Inserts in the library comprise Ig heavy chain framework regions flanking highly divergent, synthetically produced hypervariable regions. A second aspect of the invention relates to the use of single-chain recombinant binding proteins to inhibit the activity of an intracellular constituent. In the exemplary case presented, the activity of intracellular glucose-6-phosphate dehydrogenase was inhibited by intracellular expression of a cloned single-chain recombinant binding protein.

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1995:24041 Document No.: PREV199598038341. Gene therapy in malignant mesothelioma. Mew, Daphne J.; **Mulligan-Kehoe, Mary Jo**; Pass, Harvey I.. Surg. Radiat. Oncol. Branches, Natl. Cancer Inst., Natl. Inst. Health, Bethesda, MD, USA. Surgical Forum, (1994) Vol. 45, No. 0, pp. 495-499.
CODEN: SUFOAX. ISSN: 0071-8041. Language: English.

=>

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	216.16	216.37

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-16.79	-16.79

STN INTERNATIONAL LOGOFF AT 14:57:08 ON 09 SEP 2005